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Original article

Weak Anti-inflammatory and Anti-cancer Properties of Saffron

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Abstract

Background and objectives: Positive role of saffron in human health promotion has been investigated in widespread researches. Anticancer property, neuroprotection, protection of cardiovascular system and several positive properties are reported for saffron customers. The aim of this study was assessment of saffron weakness against light damage in rat retina. **Methods:** Gene profiles of control samples (C group) and light damage (L) groups were extracted from Gene Expression Omnibus (GEO) and compared with similar samples in the presence of saffron. The unprotected differentially expressed genes (DEGs) were evaluated via network analysis and pathway investigation. The critical genes which were not protected by saffron were identified and discussed. **Results:** Numbers of 67 DEGs were investigated via protein-protein interaction (PPI) network analysis, pathway assessment, and action map investigation. Findings indicated that STAT1, JUN, FOS, and STAT3 were the crucial genes that were not protected by saffron against light damage in rat retina. **Conclusion:** It may be necessary that consumption of saffron require a suitable protocol to avoid from possible disadvantages; however, saffron is well known for its benefits in human nutrition.

Keywords: *Crocus sativus*; gene expression; saffron

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Introduction

Iridaceae family comprises more than 60 genera and 800 species which grow in tropical and temperate regions. Saffron (*Crocus sativus* L.), the famous flavoring plant, is a perennial member of this family which is cultivated in Iran, India, Italy, Spain, Greece, and Morocco [1,2]. Saffron has been used as a food additive for centuries which is presumed to admit its safety for most people; however, its safety and toxicity have been the focus of several studies [3].

Total pigments of saffron have been reported to include phytoene, phytofluene, tetra-hydrolycopene, b-carotene, x-carotene, zeaxanthin, lycopene and crocin; but the major coloring agent is crocin, the digentiobioside ester of apocarotenic acid (crocetin). The special taste of saffron is due to the presence of picrocrocin and safranal. A hypothetical compound named protocrocin of the fresh plant is supposed to decompose on drying into crocin and picrocrocin.

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Hydrolysis of crocin gives gentiobiose and crocetin, while picrocrocin yields glucose and safranal. Safranal produces the characteristic aroma and together with picrocrocin the taste of saffron. Several nutraceutical properties have been reported for crocins which include antioxidant, hypolipidemic, neuroprotective, antidepressive, hypocholesterolemic, antitumor, and anticarcinogenic activities [1,4].

Anti-tumor and anti-cancer activity in different types of cancers like lung, breast, skin and prostate cancer and also leukemia have been reported for saffron. These activities have been attributed to various mechanisms including apoptosis induction, cell cycle arrest, expression of matrix metalloproteinase suppression, detoxifying enzymes modulation and decreasing the expression of inflammatory molecules [5].

Investigations indicate that saffron has a prominent role in human health promotion. There are several documents about the positive role of saffron in promotion of human health in the cases of anxiety, cardiovascular disease, gastric disorders, depression, insulin resistance, premenstrual syndrome, insomnia, and cancers [6,7]. There are evidences that saffron has a protective effect on mammalian retina against light damage [8]. The neuroprotective effect of saffron on retina is compared with photobiomodulation [9].

While positive role of saffron in human health promotion is emphasized in many investigations, there are evidences about disadvantages of saffron consuming especially in high dosage. As it is reported, high dosage consumption of saffron in rat caused decreasing the seminiferous duct thickness and germinal cells number [10]. It seems that better understanding of molecular mechanism of saffron effects on human body can provide useful information about consuming saffron [11].

Gene expression studies in the case of classical genetics investigations and also in the format of high throughput methods are known as useful tool to analyze food and supplementation consuming [12-14]. Several gene expression assessment about saffron consuming have been recorded; R Natoli et al. published an article about effects of saffron on regulation of genes and noncoding RNAs in rats in 2010 [15]. Anticancer properties of saffron, pro-inflammatory cytokine regulation by saffron, and regulation of several enzyme related to the diabetes by saffron are studies in the protein and gene expression level [16-18].

Bioinformatics and system biology are demonstrated as useful abilities to interpret high throughput data in biology and medicine [19,20]. The large numbers of data such as proteins, genes, and metabolites are analyzed to find the critical individuals which play crucial role in onset or progress of diseases or prevent damages [21,22]. In the present study, disability of saffron in protection of rat retina against light damage is evaluated to show clear prospective of saffron properties and its role in human health.

Materials and Methods

Ethical considerations

This study is originated from project with ethical code: IR.SBMU.REC.1398 confirmed by ethical committee of Shahid Beheshti University of Medical Sciences.

Data collection

For assessment of light damage in rat retina, three samples (GSM563898-900) as control group (C) and GSM563907-9 as light damage group (L) were selected from GSE22818 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22818) which is recorded as "Comparison of saffron and photobiomodulation on the light damaged rat retina" in GEO. The rats raised in dim cyclic illumination (12 h 5 lux, 12 h darkness) as C group and the challenged rats by 24 h exposure to 1,000 lux light (L group) were regarded for a comparative study. To find saffron effect, GSM563904-6 and GSM563913-15 were considered as saffron (S) group and saffron-light (SL) group. The S group were treated with 1 mg/kg/day for 3 weeks of saffron and the SL group were pretreated with 1 mg/kg/day for 3 weeks of saffron then exposed to 24 h 1000 lux light. Two set of analyses were conducted; comparative analysis of eyes gene expression profiles of "C and L groups" and "S and SL groups". Full description of experimental set up is available in the original document which is published entitle "Gene and noncoding RNA regulation underlying photoreceptor protection: microarray study of dietary antioxidant saffron and photobiomodulation in rat retina" [15].

Analyses were validated by box plot matching by use of GEO2R software. Numbers of top 250 DEGs based on p-valve (small to large) for both analyses were obtained by GEO2R. To find the

significant DEGs fold change>2 and p-value≤0.01 were considered and the characterized DEGs were determined. The common DEGs between the two analyses were considered as the light damages that were not compensated by saffron. These genes included in a PPI network by using Cytoscape 3.7.1 software via protein query of STRING database [23]. The hub nodes of main connected component of the analyzed PPI network were identified.

Biochemical pathways which were related to the nodes of main connected component were determined from Kyoto Encyclopedia of Genes and Genomes (KEGG) database by using ClueGO plugin of Cytoscape [24]. CluePedia was applied to assessment of actions such as activation, inhibition, and expression between elements of the main connected component [25]. The DEGs that involved in the three types of actions were determined to find the critical genes.

Results and Discussion

Distribution of gene expression profiles of S and SL groups were statistically matched. Since the profiles were median centered (see figure 1), the samples are comparable. Among 250 downloaded DEGs 109 individuals were characterized and valid with p-value<0.01 and FC>2. Numbers of 105 significant DEGs in the middle of the 109 individuals were recognized with STRING database and were included in the network by Cytoscape software. As it is shown in figure 2 the PPI network including 36 isolated DEGs, two paired gens and a main connected component containing 67 nodes was constructed. Since hub nodes are the important central nodes; the top 10 nodes including Stat3, Stat1, Ccl2, Fos, Icam1, Cxcl10, Ptgs2, Atf3, Jun, and Timp1 based on degree value were determined as hubs. The central properties of hub nodes including betweenness centrality, closeness centrality, degree, and stress are tabulated in table 1. The five clusters including 18 biochemical pathways related to the 67 nodes have been presented in figures 3, 4, and table 2.

Action maps including activation, inhibition, and expression actions were illustrated for the 67 DEGs to find regulatory roles of the studied genes.

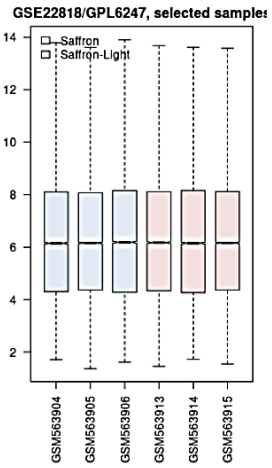

Figure 1. Box plot analysis of distribution of gene expression profiles for S and SL groups. Data are median centered

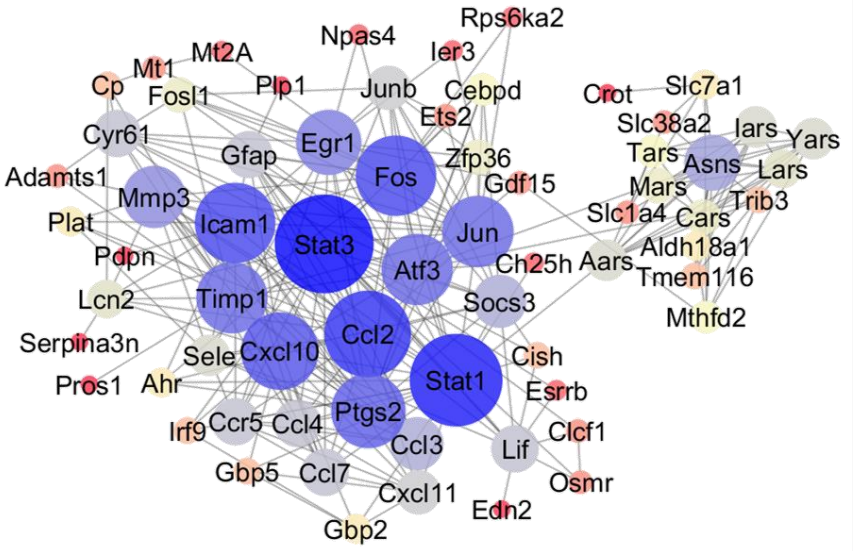

Figure 2. Main connected component of PPI network related to the 67 significant DEGs. The nodes are layout based on degree value; the bigger size and also blue color refer to the more value of degree.

Table 1. List of hub nodes of constructed network; description is obtained from STRING database and is summarized. DN, BC, CC, D, and S refer to the display name, betweenness centrality, closeness centrality, degree, and stress respectively.

DN	Description	BC	CC	D	Stress
Stat3	Signal transducer and activator of transcription 3 (acute-phase response factor); Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Acts as a regulator of inflammatory response by regulating differentiation of naive CD4 (+) T-cells into T-helper Th17 or regulatory T-cells (Treg). Involved in cell cycle regulation. Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role. Plays a crucial role in basal beta cell functions, such as regulation of insulin secretion (By similarity).	0.136	0.56	28	2220
Stat1	Signal transducer and activator of transcription 1	0.207	0.56	26	3200
Ccl2	Immediate-early serum-responsive protein JE; Chemotactic factor that attracts monocytes, but not neutrophils; Belongs to the intercrine beta (chemokine CC) family.	0.054	0.54	24	1516
Fos	FBJ osteosarcoma oncogene; It regulates TGF-beta- mediated signaling. It has a critical function in regulating the development of cells destined to form and maintain the skeleton. It is thought to have an important role in signal transduction, cell proliferation and differentiation.	0.110	0.52	22	1810
Icam1	Intercellular adhesion molecule 1; During leukocyte trans- endothelial migration, ICAM1 engagement promotes the assembly of endothelial apical cups; Belongs to the immunoglobulin superfamily.	0.055	0.50	22	1174
Cxcl10	10 kDa interferon gamma-induced protein; In addition to its role as a pro-inflammatory cytokine, may participate in T-cell effector function and perhaps T-cell development.	0.035	0.47	21	736
Ptgs2	Prostaglandin-endoperoxide synthase 2; Converts arachidonate to prostaglandin H2 (PGH2), a committed step in prostanoid synthesis. Constitutively expressed in some tissues in physiological conditions, such as the endothelium, kidney and brain, and in pathological conditions, such as in cancer. PTGS2 is responsible for production of inflammatory prostaglandins. Up-regulation of PTGS2 is also associated with increased cell adhesion, phenotypic changes, resistance to apoptosis and tumor angiogenesis. In cancer cells, PTGS2 is a key step in the production of prostaglandin E2 (PGE2), which plays important roles in modulating motility, proliferation and resistance to apoptosis; Belongs to the prostaglandin G/H synthase family.	0.024	0.52	20	806
Atf3	Cyclic AMP-dependent transcription factor ATF-3; In solution, it binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters but also has affinity for related sites. May have a unique and critical role in growth regulation of regenerating liver and mitogen-stimulated cells.	0.253	0.56	19	3840
Jun	V-jun avian sarcoma virus 17 oncogene homolog; Promotes activity of NR5A1 when phosphorylated by HIPK3 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation. Involved in activated KRAS-mediated transcriptional activation of USP28 in colorectal cancer (CRC) cells. Binds to the USP28 promoter in colorectal cancer (CRC) cells.	0.037	0.51	19	1058
Timp1	Tissue inhibitor of metalloproteinases 1; It functions as a growth factor that regulates cell differentiation, migration and cell death and activates cellular signaling cascades via CD63 and ITGB1. Plays a role in integrin signaling. Also stimulates steroidogenesis by Leydig and ovarian granuloma cells.	0.076	0.51	19	1516

As depicted in figures 5-7, considerable numbers of DWGs participated in the action maps. Activation contains most relationship between the connected genes and inhibition has limited participant. To find the critical genes which are involved in the action maps, relationships between the DEGs that are linked with the other genes by all types of connections have been determined and shown in figure 8.

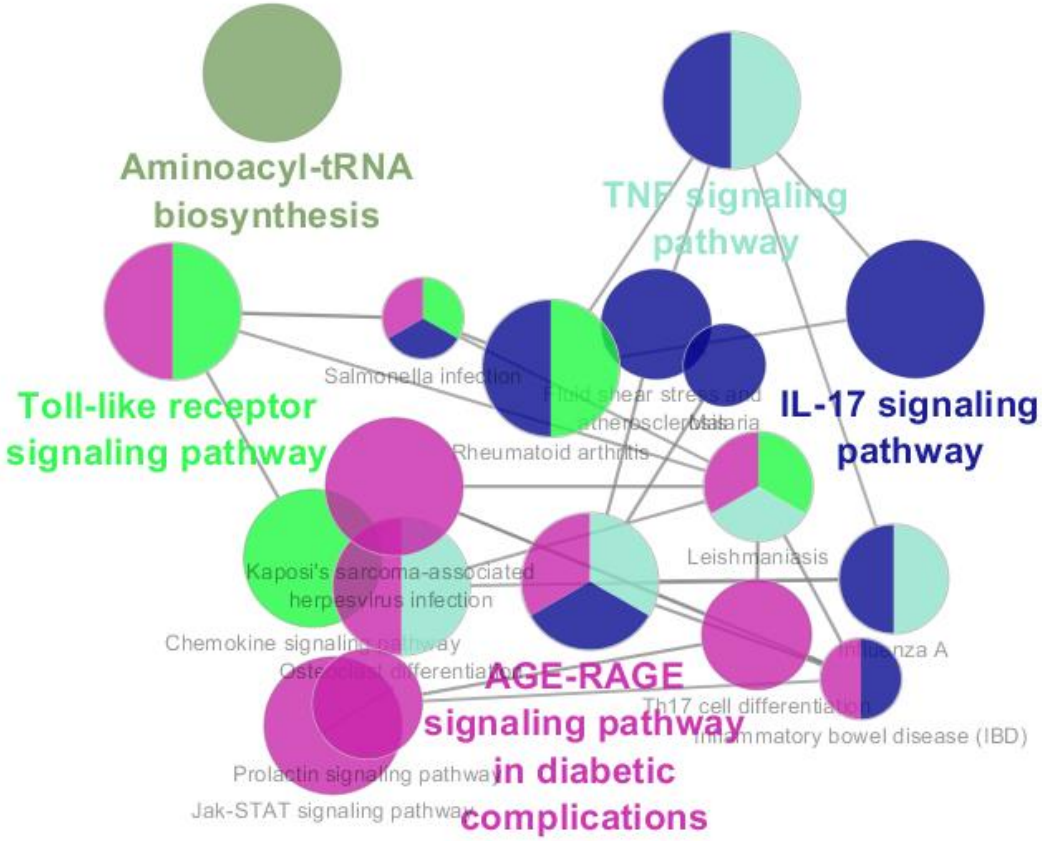

Figure 3. The 5 clusters including 18 biochemical pathways related to the 67 DEGs. Members of each cluster are shown in a similar color.

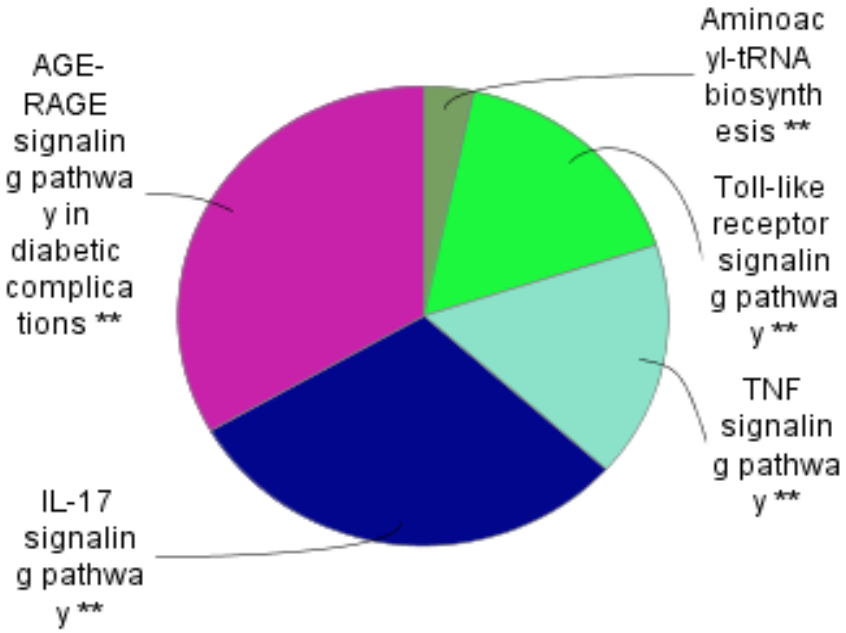

Figure 4. The 5 clusters of biochemical pathways related to the 67 DEGs. The colored areas refer to the frequency of the numbers of pathways which are included in the corresponded clusters; p-value≤0.01 was considered.

Table 2. Five clusters including 18 biochemical pathways related to the 46 DEGs. The pathways were obtained from KEGG and group p-value corrected with bonferroni step down and was less than 0.01. R, Gog, GO TERM, %AG, and NG refer to Row, gene ontology group, gene ontology term, % associated genes, and No. of genes respectively.

R	GOg	GO Term	% AG	NG	Associated Genes Found
1	1	**Aminoacyl-tRNA biosynthesis**	11	7	[Aars, Cars, Iars, Lars, Mars, Tars, Yars]
2	2	Chemokine signaling pathway	5	9	[Ccl2, Ccl3, Ccl4, Ccl7, Ccr5, Cxcl10, Cxcl11, Stat1, Stat3]
3		**Toll-like receptor signaling pathway**	7	7	[Ccl3, Ccl4, Cxcl10, Cxcl11, Fos, Jun, Stat1]
4		Salmonella infection	5	4	[Ccl3, Ccl4, Fos, Jun]
5		Leishmaniasis	6	4	[Fos, Jun, Ptgs2, Stat1]
6		Rheumatoid arthritis	7	6	[Ccl2, Ccl3, Fos, Icam1, Jun, Mmp3]
7	3	Osteoclast differentiation	5	7	[Fos, Fosl1, Irf9, Jun, Junb, Socs3, Stat1]
8		**TNF signaling pathway**	10	11	[Ccl2, Cxcl10, Fos, Icam1, Jun, Junb, Lif, Mmp3, Ptgs2, Sele, Socs3]
9		AGE-RAGE signaling pathway in diabetic complications	7	7	[Ccl2, Egr1, Icam1, Jun, Sele, Stat1, Stat3]
10		Leishmaniasis	6	4	[Fos, Jun, Ptgs2, Stat1]
11		Influenza A	4	7	[Ccl2, Cxcl10, Icam1, Irf9, Jun, Socs3, Stat1]
12	**4**	**IL-17 signaling pathway**	10	9	[Ccl2, Ccl7, Cxcl10, Fos, Fosl1, Jun, Lcn2, Mmp3, Ptgs2]
13		TNF signaling pathway	10	11	[Ccl2, Cxcl10, Fos, Icam1, Jun, Junb, Lif, Mmp3, Ptgs2, Sele, Socs3]
14		AGE-RAGE signaling pathway in diabetic complications	7	7	[Ccl2, Egr1, Icam1, Jun, Sele, Stat1, Stat3]
15		Salmonella infection	5	4	[Ccl3, Ccl4, Fos, Jun]
16		Malaria	6	3	[Ccl2, Icam1, Sele]
17		Influenza A	4	7	[Ccl2, Cxcl10, Icam1, Irf9, Jun, Socs3, Stat1]
18		Inflammatory bowel disease (IBD)	5	3	[Jun, Stat1, Stat3]
19		Rheumatoid arthritis	7	6	[Ccl2, Ccl3, Fos, Icam1, Jun, Mmp3]
20		Fluid shear stress and atherosclerosis	4	6	[Ccl2, Fos, Icam1, Jun, Plat, Sele]
21	5	Osteoclast differentiation	5	7	[Fos, Fosl1, Irf9, Jun, Junb, Socs3, Stat1]
22		Toll-like receptor signaling pathway	7	7	[Ccl3, Ccl4, Cxcl10, Cxcl11, Fos, Jun, Stat1]
23		Jak-STAT signaling pathway	5	8	[Cish, Gfap, Irf9, Lif, Osmr, Socs3, Stat1, Stat3]
24		Th17 cell differentiation	5	5	[Ahr, Fos, Jun, Stat1, Stat3]
25		Prolactin signaling pathway	7	5	[Cish, Fos, Socs3, Stat1, Stat3]
26		**AGE-RAGE signaling pathway in diabetic complications**	7	7	[Ccl2, Egr1, Icam1, Jun, Sele, Stat1, Stat3]
27		Salmonella infection	5	4	[Ccl3, Ccl4, Fos, Jun]
28		Leishmaniasis	6	4	[Fos, Jun, Ptgs2, Stat1]
29		Kaposi's sarcoma-associated herpesvirus infection	4	9	[Ccr5, Fos, Icam1, Irf9, Jun, Ptgs2, Stat1, Stat3, Zfp36]
30		Inflammatory bowel disease (IBD)	5	3	[Jun, Stat1, Stat3]

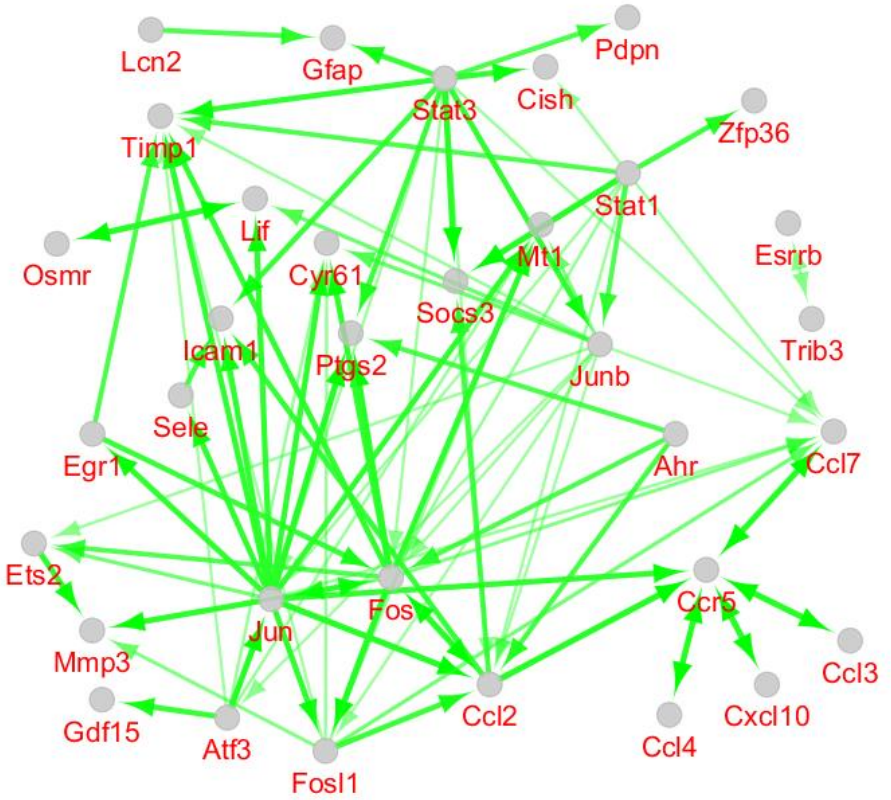

Figure 5. Activation actions between 67 DEGs is presented. Among 67 genes, 34 individuals were connected; directions of arrows refer to the activation

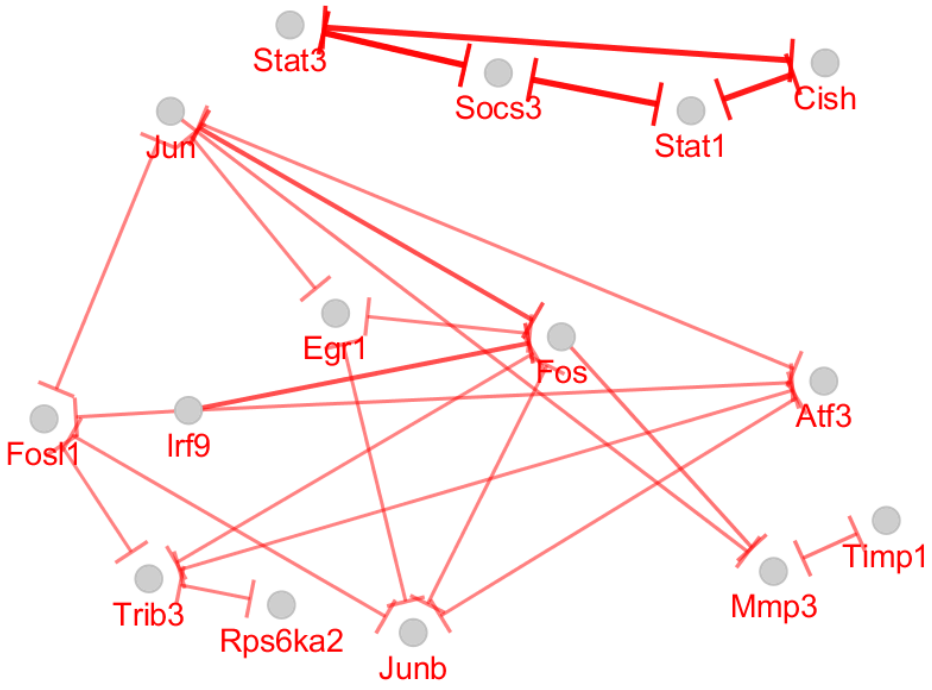

Figure 6. Inhibition actions between 67 DEGs is presented. Among 67 genes, 15 individuals were connected; Bar tips of connections refer to the inhibition

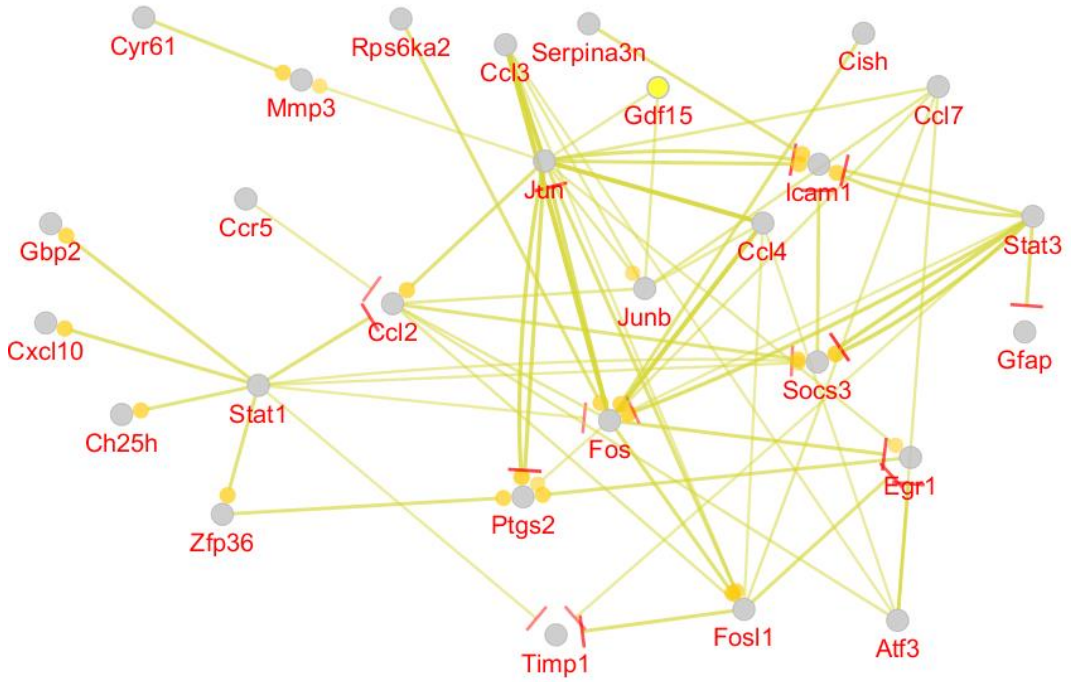

Figure 7. Expression actions between 67 DEGs is shown. Among 67 genes, 28 individuals were connected; bar and round tips of connections refer to the down and up-regulation relationships

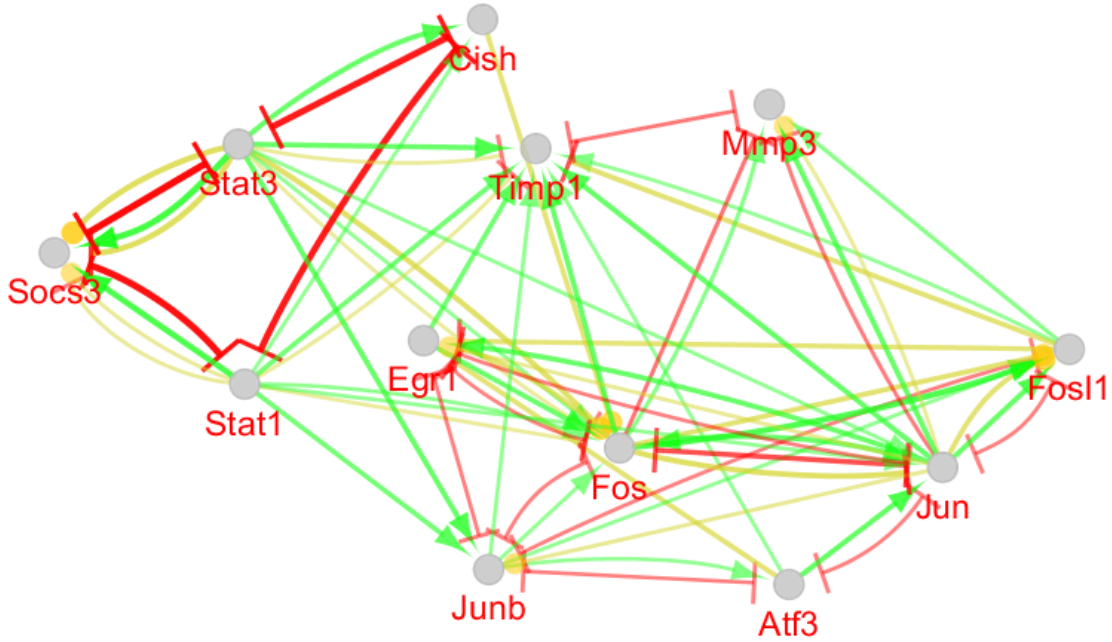

Figure 8. Genes among 67 DEGs which are connected with the other genes by all types of contacts. Green, red, and yellow colors refer to activation, inhibition, and expression respectively.

As it is shown in the figure 1, the samples were matched statistically and distribution of gene expression amounts was comparable. As it is shown in the results part; 5 methods were designed to evaluate the deregulated DEGs. In the first attempt the 105 significant DEGs that were not protected by saffron versus light damage in retina were determined. The second screening was done via network construction; 67 DEGs were highlighted as interacted individuals. Schema in figure 2 refers to a scale free network which provides feasibility to introduce the limited key nodes as central nodes [26]. The 67 significant DEGs were analyzed to find the central genes in the third step of study. Numbers of 10 hubs are listed in table 1 that probably are the key genes which were not protected by saffron against light damage in retina. In the fourth stage of investigation; five clusters of biochemical pathways including 18 pathways related to the 67 queried DEGs were determined (figures 3,4, and table 2). In the fifth part of the study; action roles of the interacted 67 DEGs were assessed to find the regulatory character of the studied genes. The final finding was formed from the merged results of the mentioned five stages of study.

As it is shown in figure 1, two parts of the network were formed with 50 and 17 nodes. All central nodes were present in the larger part of the network. Regarding betweenness centrality, there were 4 hub-bottlenecks including STST3, STAT1, FOS, and ATF3 (see table 2). The hub-bottleneck nodes also are presented in the large part of the network. It is a reasonable conclusion that the critical genes are involved in the large part of the network.

Since gene ontology is a suitable method to explain roles of the studied genes; the 28 genes that are involved in the 18 biochemical pathways have been tabulated in table 2. Among 28 genes 6, 4, 3, 1, 2, 1, and 3 genes are involved in 1, 2, 3, 4, 5, 6, and 7 pathways respectively. The other remained genes are JUN, STAT1, FOS, CCL2, ICAM1, STAT3, CCL3, and CXCL10 that are related to the 23, 18, 15, 13, 12, 10, 8, and 8 pathways respectively. It seems this set of 8 genes can be considered as critical genes beside the introduced hub genes. A simple matching shows that except CCL3, the other 7 critical involved genes in the pathways are common with the hub nodes.

Action analysis (figures 5-8) led to introducing 12 genes that play role in the three studied actions; activation, inhibition, and expression. JUN, STAT1, FOS, and STAT3 are the common genes between action analysis and results of centrality and pathway assessment. Therefore it can be concluded that non-protective property of saffron against light damage mostly is related to the dys-regulation of these 4 crucial genes.

Description of the 4 highlighted crucial genes is recorded in table 1. It seems these genes are related to the essential processes such as apoptosis, cell proliferation, inflammation, and signal transduction. Relationship between JUN and FOS as two nuclear oncoproteins is a well-known correlation [27,28] that has been investigated in cell proliferation. This finding is not consisted with the anticancer property of saffron [29]. There are many investigations about role of STAT1 in the innate immunity function. S Dupuis et al. reported that STAT1 deficiency is correlated to the lethal viral disease in human. Researches have shown that STAT1 activation is depended to many cytokines and growth factors [30,31]. Similar to STAT1, prominent role of STAT3 in the innate immunity is emphasized. It is reported that dysregulation of STAT3 in Crohns diseases is significant [32,33]. While anti-inflammatory properties of saffron are confirmed, dysregulation of STAT1 and STAT3 in the presence of saffron is a considerable finding that needs more attention.

JUN, FOS, STAT1, and STAT3 were identified as the crucial genes that were dysregulated by light damage in the rat retina in the presence of saffron. It seems that beside the useful effects of saffron on human health, other aspects of saffron consumption should be considered. The findings indicated that consumption of saffron is accompanied with complex biological effects on body. It may be necessary that negative features of saffron on the human health be controlled by definition of suitable protocols for saffron consumption via further investigations.

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Author contributions

Mohammah Hossein Heidari, Zahra Razzaghi, Mohammad Rostami-Nejad, Sina Rezaei-Tavirani, and Saeed Safari contributed to the data

gathering, writing of manuscript, and data analysis. Mostafa Rezaei-Tavirani and all of the authors designed project and participated in editing of 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

GEO: Gene Expression Omnibus; DEGs: differentially expressed genes; PPI: protein-protein interaction; KEGG: Kyoto Encyclopedia of Genes and Genomes; FC: fold change; DN: display name; BC: betweenness centrality; CC: closeness centrality; Gog: gene ontology group; GO term: gene ontology term; AG: associated genes