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Original article

Assessment of Saffron Neuroprotective Properties in Rat Retina versus Light Damage

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Abstract

Background and objectives: *Crocus sativus* L. (Iridaceae) commonly known as saffron, is a popular spice which is used for its pleasant aroma and favored color. Regarding the previous reports about the neuroprotective behavior of saffron or its constituents, in the present work, the neuroprotective property of saffron in rat retina was investigated against light damage in a system biology study. **Methods:** Retina gene profiles of 4 groups (each group including 3 samples) of rats (control; C light damage; L, Saffron; S, and saffron-light damage; SL) which are included in GSE22818 were extracted from Gene Expression Omnibus (GEO). The significant differentially expressed genes (DEGs) from C-L groups analysis which are not included in S-SL comparison were screened by pathway analysis to find the critical protected genes against light damage by saffron. **Results:** Numbers of 46 gene were protected by saffron versus light damage significantly. The findings revealed that Casp3, Myd88, Birc3, Tnfrsf1a, Myc, Nfkb2, Fgf2 were the important protected genes by saffron against light damage. "MAPK signaling pathway" and "apoptosis" were highlighted as important related pathways for 46 DEGs. **Conclusion:** Saffron protects a part of light damage which is controlled mostly by Casp3, Myd88, Birc3, Tnfrsf1a, Myc, Nfkb2, Fgf2. It seems other parts of damage should be studied in more details to find a complete prospective of molecular mechanism of light damage effect on retina.

Keywords: *Crocus sativus*; genetic association studies; rats; retina; saffron

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Introduction

Nine species of *Crocus* (Iridaceae) grow in Iran. C*rocus sativus* L. (saffron) is known as one of the

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most important plants for culinary use over the world [1]. The dried stigmas of the species being one of export commodities of Iran are used as tonic, for improving mood and also as a flavoring agent because of its coloring property and pleasant aroma [2]. Crocetin esters, picrocrocin and safranal being the main constituents of saffron, are accompanied by about 150 other volatile and nonvolatile components in the stigmas such as carbohydrates, proteins, fats, anthocyanins, flavonoids, vitamins minerals and many other elements [3]. Crocin produces the specific known color of saffron while picrocrocin the flavor and safranal the aroma which are synthesized during the flowering stage through cleavage of zeaxanthin, followed by oxidative modifications and glycosilations [4].

The first document about application of saffron in medicine is found in Assurbanipal library [5]. There are several documents about the role of saffron in treatment of various diseases such as Alzheimer's disease, cardiovascular disease, and skin diseases [6-8]. The neuroprotective effects of saffron have been the focus of many studies regarding the neurodegenerative diseases. A number of previous studies believed that the effects were the result of the antioxidant potential of the plant [9], but new evidences show modulating the brain transcriptome in the absence of an insult [10]. Saffron has also showed to increase differentiation of neural stem cells into oligodendrocytes [11]. In a previous in vivo study, safranal neuroprotective effects have been reported in ischemic reperfusion injury in the rat model of stroke. The effects were attributed to the antioxidant activity [12].

Neuroprotection of saffron in retina has been investigate previously [13]; while, the anti-cancer property of the extract has been reported in 2011 [14]. Mechanism of anticancer property of crocin (the main carotenoid of saffron) has been investigated and discussed with details [15].

High throughput methods are useful tools to investigate molecular mechanism of medical interventions, nutrition and food sciences and pharmacological investigations [16,17]. In such approaches, expression changes of total genes, proteins, RNAs, or metabolites are assessed to find wide ranges of molecular alterations after application of interventions [18]. Analysis and management of data in these methods require complex exploration and interpretation. System biology and bioinformatics are used widely to find clear prospective about such findings [19]. Gene ontology can provide useful information about molecular function, biological processes, cellular component, and biochemical pathways that are related to the studied genes. Many diseases and clinical interventions are studied via gene ontology analysis. Examples include pathway analysis of several types of cancers such as kidney, breast, and ovarian cancers [20-22].

Critical genes which have been involved in the gastric cardia adenocarcinoma were screened via pathway analysis in 2013 [23]. Also, our previous investigations via pathway analysis have led to new molecular mechanism prospectives for gastric atrophy [23,24]. Rostami-Nejad et al., via pathway analysis, showed that dysregulation of cytokine-mediated signaling pathway is a critical process after laser therapy of skin [25]. In the present study, protective properties of saffron against light damage in rat retina have been studied via gene ontology and the critical protected genes and the relevant biochemical pathways were assessed.

Materials and Methods

Ethical considerations

This study is originated from project with ethical code: IR.SBMU.REC.1398.154.

Data collection

Data was obtained from GEO. GSE22818 which is recorded as "Comparison of Saffron and Photobiomodulation on the light damaged rat retina". Data is related to the published article entitled "Gene and noncoding RNA regulation underlying photoreceptor protection: microarray study of dietary antioxidant saffron and photobiomodulation in rat retina" [26]. Two sets of GSMs (GSM563898-900 and GSM563907-9 as normal and light-damage samples, respectively) were nominated to be analyzed. The control (C) samples (albino Sprague Dawley rats) were experienced 12h 5 lux, 12h darkness in a cyclic illumination while the light-damage (L) sample exposed 24h 1000 lux light. Another two sets of GSMs (GSM563904-6 and GSM563913-5) were designated for a comparative study. GSM563904-6 were the samples that were treated with 1 mg/kg/day for 3 weeks of saffron as saffron

(S) group. The saffron-light damage (SL) group was the GSM563913-5 which were treated with 1 mg/kg/day for 3 weeks of saffron then exposed to 24h 1000 lux light. RNAs of 1 eye of each rat in the group were hybridized to Affymetrix rat genome ST arrays [26].

Bioinformatics analysis

To validate the comparative study, the retina gene expression profiles of C and L groups and also S and SL groups were matched via box plot analysis by use of GEO2R software. By using "top 250" option of GEO2R, 250 top DEGs based on p-valve (small to large) were extracted and fold change>2 and p-value≤0.01 were considered. Adjusted p-value was less than 0.1. Finally, the characterized individuals were selected as significant DEGs. The DEGs that were appeared in the C-L analysis and were not included in the S-SL comparison, were considered as the protected genes against light by saffron.

Gene ontology of these genes was investigated via ClueGO application of Cytoscape software [27]. The relevant biochemical pathways were determined from Kyoto Encyclopedia of Genes and Genomes (KEGG) database and were clustered [28]. Kappa score ≥ 0.5 was regarded. for this analysis was less than 0 Action roles of genes including activation, inhibition, expression, and binding depending on involvement of gene in the determined pathways were assessed by CluePedia plugin of Cytoscape software [29]. Based on action map analysis, the crucial genes were identified and their important protective roles against light damage were discussed.

Results and Discussion

Numbers of 119 and 109 significant DEGs were determined for C-L analysis and S-SL comparison, respectively. There were 73 common DEGs between the two examinations. There were 46 DEGs in C-L analysis which were not seen in S-SL comparison and have been shown in table 1. As it is shown in the table 1, there were two isoforms of lad1 with near fold change and most of the identified DEGs were up-regulated.

The activation map related to the biochemical pathways for the 46 protected genes has been shown in figure 1. Numbers of 17 pathways that are clustered in the 4 groups including MAPK signaling pathway, breast cancer, TNF signaling pathway, and apoptosis were identified from KEGG. These groups of pathways and the relevant genes have been shown in table 2. As it is shown in figure 1, among the 46 genes 18 have been recognized by CluePedia and 13 individuals including Lif, Nfkb2, Irf1, Myd88, Birc3, Esr2, Tnfrsf1a, Myc, Epha2, Csf1, Casp3, Fgf2, and Serpine1 were acted via activation action.

Inhibition map has been shown in figure 2. As it is shown in this figure, Birc3, Casp3, Esr2, Myc, Tnfrsf1a, Nfkb2, Myd88, Epha2, and Fgf2 were involved in inhibition action. While Hspb1, Epha2, Fgf2, Myc, Nfkb2, Csf1, and Myd88 were highlighted based on expression action, Myd88, Irf1, Esr2, Epha2, Fgf2, Birc3, Tnfrsf1a, and Casp3 have been pointed in the binding map (see figures 3 and 4). For better understanding of action roles of the 18 introduced genes, their actions have been summarized in the table 3.

The results indicated that there are 46 significant DEGs that are protected by saffron against light damage. Since the aim of this study was screening of the introduced DEGs to find the critical individuals, pathway analysis was selected as screening tool. Pathway analysis is a well-known method to understand biological prospective of differential expression events [30,31]. As it is presented in table 1, 46 genes are protected against light damage by saffron. Benefit of saffron supplementation on retinal flicker sensitivity improvement has been reported by M Piccardi et al. [32]. The top 10 DEGs based on fold change are Hspb1, Lif, Hmox1, Ccl12, Npas4, Lad1, Fgf2, Lad1, Nfkb2, and Lrrc15. Biochemical pathway analysis revealed that among these 10 DEGs, Hspb1, Lif, Ccl12, Fgf2, and Nfkb2 are involved in the 17 introduced pathways. Beside these 4 DEGs, 14 other genes were related to the identified pathways. It seems that protection of a set of genes including these 18 highlighted genes, is the core of saffron effect on retina.

Action maps analysis showed that each one of these 18 critical genes plays different regulatory role. While Selp, Il17rb, Gadd45g, and Ccl12 do not participate as a regulator, Myd88, Epha2, and Fgf2 play as regulator in activation, inhibition, expression actions and also issued binding property. Based on result of table 3, Casp3 is related to the 13 pathways while Lif , Irf1, Il17rb, Selp, Epha2, Hspb1, and Esr2 are involved in the 1 pathway separately.

Presence of genes in the identified pathways also is not similar; there are 10 genes which are related to the "MAPK signaling pathway" even though there are 9 pathways with 3 relevant genes.

Table 1. The 46 protected genes against light by saffron. Fold changes of all genes have been characterized by p<0.001. The five down-regulated genes have been asterisked; the other 41 individuals have been up-regulated

NO	Gene symbol	Gene title	FC	Adj. p-value
1	Hspb1	Heat shock protein family B (small) member 1	6.0	0.096
2	Lif	Leukemia inhibitory factor	5.8	0.018
3	Hmox1	Heme oxygenase 1	5.3	0.051
4	Ccl12	Chemokine (C-C motif) ligand 12	5.0	0.076
5	Npas4	Neuronal PAS domain protein 4	4.1	0.096
6	Lad1	Ladinin 1	3.9	0.033
7	Fgf2	Fibroblast growth factor 2	3.9	0.007
8	Lad1	Ladinin 1	3.2	0.044
9	Nfkb2	Nuclear factor kappa B subunit 2	3.2	0.047
10	Lrrc15	Leucine rich repeat containing 15	3.2	0.041
11	Birc3	Baculoviral IAP repeat-containing 3	2.9	0.096
12	Mlf1	Myeloid leukemia factor 1	2.9	0.101
13	Casp3	Caspase 3	2.9	0.016
14	Chi3l1	Chitinase 3 like 1	2.8	0.065
15	Trpm2*	Tribbles pseudokinase 3	2.7	0.027
16	Epha2	Eph receptor A2	2.7	0.007
17	Adamts1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	2.6	0.019
18	Myc	Myelocytomatosis oncogene	2.6	0.049
19	Chst3	Carbohydrate sulfotransferase 3	2.6	0.044
20	Upp1	Tubulin, beta 6 class V	2.6	0.068
21	Selp	Selectin P	2.6	0.083
22	Irf1	Interferon regulatory factor 1	2.5	0.046
23	Il17rb	Interleukin 17 receptor B	2.5	0.028
24	Aqp1*	Aquaporin 1	2.5	0.014
25	Slc26a8	Solute carrier family 26 member 8	2.4	0.044
26	Cd44	CD44 molecule (Indian blood group)	2.4	0.096
27	Myd88	Myeloid differentiation primary response 88	2.4	0.051
28	RGD1564171	Rgd1564171	2.4	0.022
29	Tgm1	Threonyl-trna synthetase	2.3	0.010
30	Csf1	Colony stimulating factor 1	2.3	0.037
31	Tnfrsf1a	Transmembrane protein 116	2.3	0.037
32	Cerk*	Ceramide kinase	2.3	0.032
33	Nfkbiz	NFKB inhibitor zeta	2.2	0.046
34	Rbm41	RNA binding motif protein 41	2.2	0.049
35	Tal2	TAL bhlh transcription factor 2	2.2	0.076
36	Agt*	Angiotensinogen	2.2	0.026
37	Il6st	Interleukin 6 signal transducer	2.2	0.046
38	Gng11	Guanine nucleotide binding protein (G protein), gamma 11	2.2	0.096
39	Esr2	Estrogen receptor 2	2.2	0.083
40	Gadd45g	Growth arrest and DNA-damage-inducible, gamma	2.1	0.097
41	Ptpn1	Protein tyrosine phosphatase, non-receptor type 1	2.1	0.032
42	Sbno2	Strawberry notch homolog 2	2.1	0.059
43	Serpine1	Serpin family E member 1	2.1	0.095
44	Tubb6	Transient receptor potential cation channel, subfamily M, member 2	2.1	0.063
45	Ppef1*	Protein phosphatase with EF-hand domain 1	2.0	0.032
46	Klf11	Kruppel-like factor 11	2.0	0.032

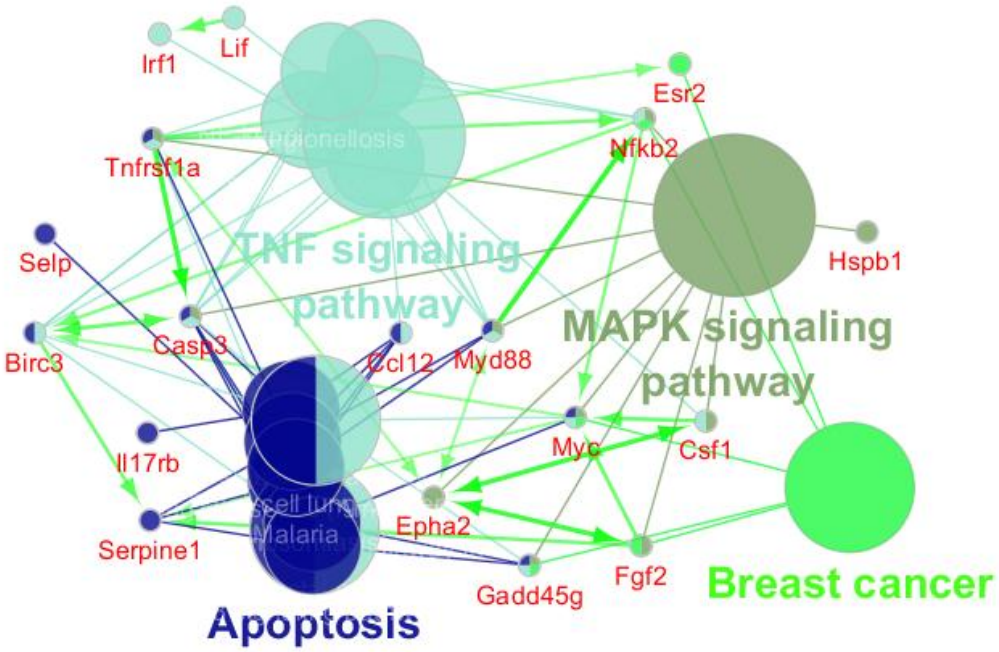

Figure 1. Four clusters of 17 pathways related to the 46 protected genes by saffron from light damage have been presented. The related genes with activation role have been presented. Among 46 genes 18 individuals (red color) were recognized by CluePedia. The green directed arrows refer to the direction of activation. The bright and dark green undirected linked refer to the genes which are directed to the "breast cancer" and "MAPK signaling pathway" pathways. Kappa score ≥ 0.5 was regarded

Table 2. Numbers of 17 Biochemical pathways related to the 46 protected genes that were included in the 4 clusters found KEGG. Term and group p-value, and term and group p-value corrected with Bonferroni step down<0.01 were considered. NO, GO TERM, %AG, and NG refer to gene ontology group, gene ontology term, % associated genes, and No. of genes, respectively; names of groups have been identified with the asterisked pathways.

NO	GO Term	% AG	NG	Associated Genes Found
1	MAPK signaling pathway*	3.41	10	[Casp3, Csf1, Epha2, Fgf2, Gadd45g, Hspb1, Myc, Myd88, Nfkb2, Tnfrsf1a]
2	Breast cancer*	3.31	5	[Esr2, Fgf2, Gadd45g, Myc, Nfkb2]
3	NF-kappa B signaling pathway	3.85	4	[Birc3, Myd88, Nfkb2, Tnfrsf1a]
	Apoptosis	9.38	3	[Birc3, Casp3, Tnfrsf1a]
	TNF signaling pathway*	5.56	6	[Birc3, Casp3, Ccl12, Csf1, Lif, Tnfrsf1a]
	Pertussis	4.00	3	[Casp3, Irf1, Myd88]
	Legionellosis	5.17	3	[Casp3, Myd88, Nfkb2]
	Toxoplasmosis	3.70	4	[Birc3, Casp3, Myd88, Tnfrsf1a]
	Small cell lung cancer	4.35	4	[Birc3, Casp3, Gadd45g, Myc]
4	p53 signaling pathway	4.48	3	[Casp3, Gadd45g, Serpine1]
	Apoptosis*	9.38	3	[Birc3, Casp3, Tnfrsf1a]
	IL-17 signaling pathway	3.30	3	[Casp3, Ccl12, Il17rb]
	AGE-RAGE signaling pathway in diabetic complications	3.00	3	[Casp3, Ccl12, Serpine1]
	Chagas disease (American trypanosomiasis)	3.96	4	[Ccl12, Myd88, Serpine1, Tnfrsf1a]
	Malaria	6.12	3	[Ccl12, Myd88, Selp]
	Colorectal cancer	4.05	3	[Casp3, Gadd45g, Myc]
	Small cell lung cancer	4.35	4	[Birc3, Casp3, Gadd45g, Myc]

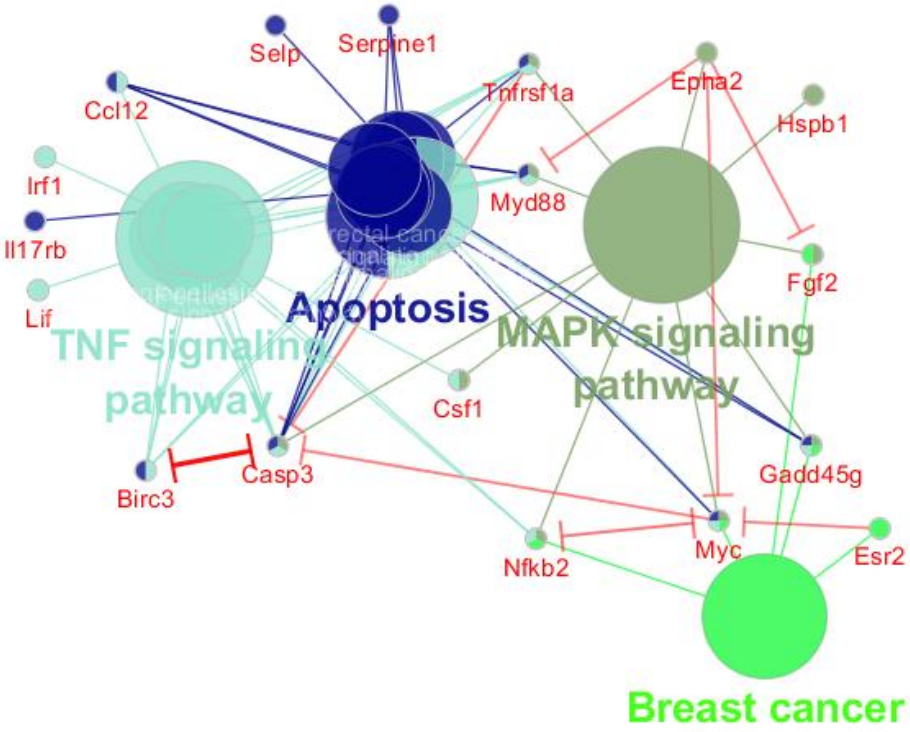

Figure 2. Inhibition map for the 18 recognized genes by CluePedia. The red directed arrows refer to the direction of inhibition. Kappa score ≥ 0.5 was regarded

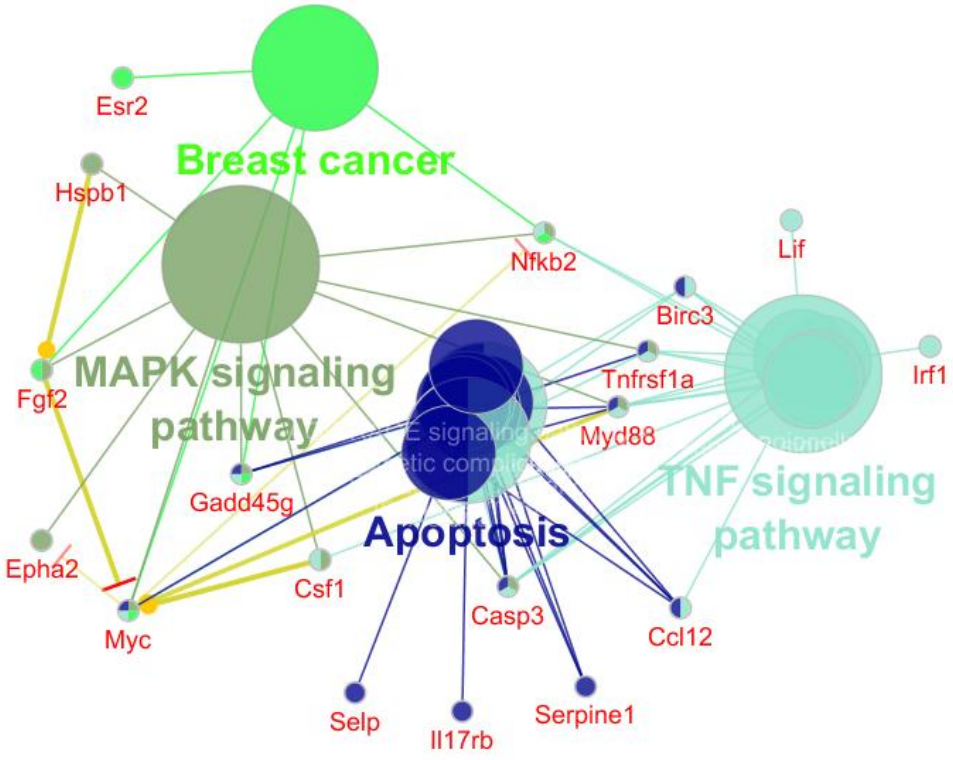

Figure 3. Expression map for the 18 recognized genes by CluePedia. The yellow arrows refer to the expression relationship. Red and round tips are corresponded to the down and up-regulation, respectively. The undirected yellow arrows refer to the correlation between expressions of the both connected nodes. Kappa score ≥ 0.5 was regarded

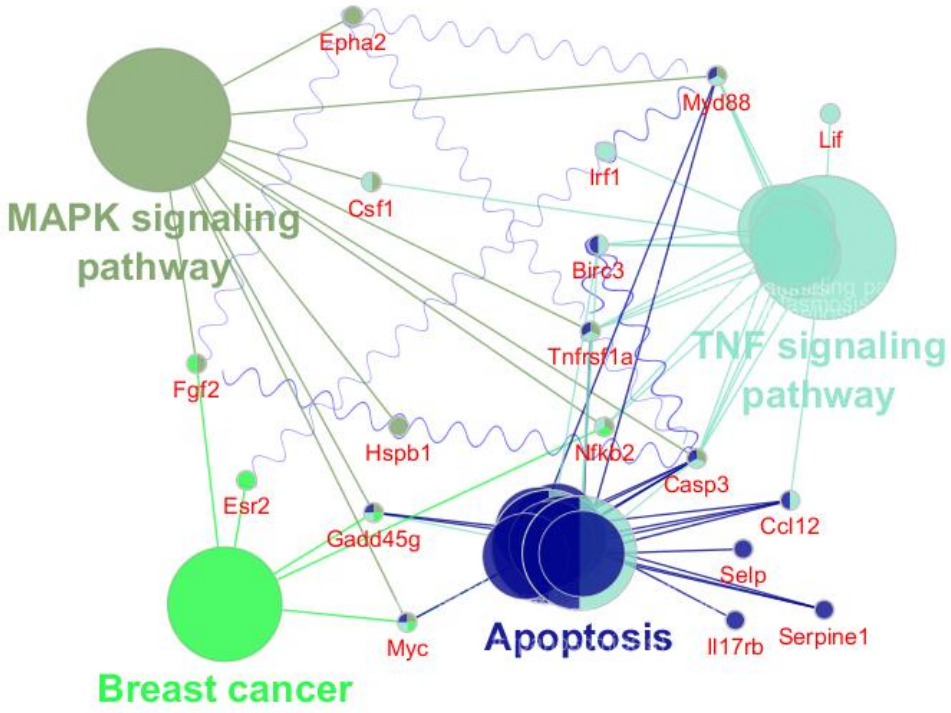

Figure 4. Binding map for the 18 recognized genes by CluePedia. The sine wave arrows refer to the binding relationships between the connected genes. Kappa score ≥ 0.5 was regarded

Table 3. Action roles of the 18 genes which were involved in the action maps

No	Gene	Activation mode		Inhibition mode		Expression role		Binding
		Activated	Activator	inhibited	inhibitor	Regulator	Regulated	
1	Lif		√					
2	Nfkb2	√	√	√	√		√	
3	Irf1	√						√
4	Myd88	√	√	√		√		√
5	Birc3	√	√	√	√			√
6	Esr2	√			√			√
7	Tnfrsf1a	√	√		√			√
8	Myc	√	√	√	√	√	√	
9	Epha2	√	√	√	√		√	√
10	Csf1	√	√			√		
11	Casp3	√	√	√	√			√
12	Fgf2	√	√	√		√	√	√
13	Serpine1	√						
14	Selp							
15	Il17rb							
16	Gadd45g							
17	Hspb1					√		
18	Ccl12							

Two conditions were considered to screen the 18 critical genes to find the more important ones; a: the genes that are related to the one pathway were excluded and b: the genes which are presented in three action modes (as it has been shown in the table 3) are included to more assessment. Based on condition a; Lif, Irf1, Il17rb, Selp, Epha2, Hspb1, and Esr2 were excluded from more investigation. Lif, Irf1, Il17rb, Selp, Hspb1, Csf1, Serpine1, Gadd45g, and Ccl12 were excluded based on condition b. Lif , Irf1, Il17rb, Selp, and Hspb1 are common between the 2 defined conditions. Merged results of the 2 conditions indicated that Casp3, Myd88, Birc3, Tnfrsf1a, Myc, Nfkb2, Fgf2 are the important protected genes by saffron against light damage.

As it is shown in the table 2, except Birc3 the other 6 important genes are related to the "MAPK signaling pathway". All 3 relevant genes (Birc3, Casp3, and Tnfrsf1a) of "apoptosis" pathway are presented in the important gens list. It seems that a panel including these 7 important genes reveals the core protective effect of saffron in retina versus light damage.

Khuloud Bajbouj et al. have reported that saffron activates Casp3 via P53-depended process in HCT116 cells [33]. Correspond to the finding of Kuloud Bajbouj et al., about 3 fold change for up-regulation of Casp3 has been recorded in the table 1. Anneli Kangas et al. confirmed activation of Casp3 via induced apoptosis by c-Myc [34]. Consistent with findings of Anneli Kangas et al. **i**ndirect activation of Casp3 by Myc via Birc3 has been illustrated in the figure 1. Suppression of Nfkb2 by Myc has been reported by Ulrich Keller et al. [35]. Inhibition and suppression of Nfkb2 by Myc were shown in the figures 2 and 3, respectively. Cliona M et al. demonstrated that Nfkb2 and Tnfrsf1a were involved in the "death receptor signaling" pathway [36] while Piccardi et al. emphasized the protective effect of saffron versus up-regulation of Fgf2 in light exposed rats [32]. Inhibition of Fgf2 and Myd88 by Epha2 has been illustrated in figure 2.

Both "MAPK signaling pathway" and "apoptosis" that appeared as the related pathways of saffron effect in this study, are two important pathways. Investigation have indicated that trouble in control of MAPK signaling pathway is accompanied with development of many disease in human such as Alzheimer's disease, several types of cancers, Parkinson's disease, and amyotrophic lateral sclerosis [37]. Importance of apoptosis in life of human and the other organisms, disease, and especially in cancer is discussed in details by many researchers [38-40].

In conclusion the findings of the present study indicate that saffron compensated a considerable part of light damage in retina of rats. The protected genes by saffron are involved in the significant pathways such as "MAPK signaling pathway" and "apoptosis"; however, this protective property of saffron versus light damage is not perfect.

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Author contributions

Mostafa Rezaei Tavirani and Nayebali Ahmadi designed and supervised the study; Mohammad Rostami-Nejad, Majid Rezaei-Tavirani, and Mohammad Hossein Heidari were involved in data collection and data analysis; Mohammadreza Razzaghi, Maryam Hamzeloo-Moghadam, and Saeed Safari participated in data analysis, and 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

C: control; L: light group; S: saffron; SL: saffron-light damage; GEO: gene expression omnibus; DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; FC: fold change; NO: gene ontology group; GO: gene ontology; %AG: % associated genes; NG: number of genes