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The Effect of the Saffron Intervention on NAFLD Status and Related Gene Expression in a Rat Model

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Abstract

Background: According to the worldwide increasing prevalence of non-alcoholic fatty liver disease (NAFLD), the present study aimed to investigate the mechanism effects of saffron consumption on preventing NAFLD in a rat model.

Methods: In an experimental study, 12 rats were randomly divided into 2 groups to be evaluated in the prevention phase for 7 weeks. In the prevention phase, the animals were randomly assigned to either fed HFHS + 250 mg/kg saffron (S) or fed with HFHS. Afterward, parts of the liver were excised for histopathologic examination. Plasma concentrations of ALT, AST, GGT, ALP, serum lipids, insulin concentrations, plasma glucose, hs-CRP, and TAC were measured. Moreover, Also, the gene expression of 6 target genes was evaluated, including FAS, ACC1, CPT1، PPARα ،DGAT2, and SREBP 1-c at the beginning and end of the study. Also, the differences among groups were evaluated by the Mann-Whitney test for non-normal data and the independent t test for normal data.

Results: The prevention phase groups have a significant elevation in body weight ($P = 0.034$) and food intake ($P = 0.001$) of the HFHS group versus HFHS + 250 mg/kg S group. Also, there was a significant difference between groups 1 and 2 for ALT (P = 0.011) and AST ($P = 0.010$), and TG ($P = 0.040$). The HFHS group had higher plasma levels of FBS ($P = 0.001$), insulin ($P = 0.035$), HOMA-IR ($P = 0.032$), and lower TAC ($P = 0.041$) versus the HFHS+ S group. Also, the difference between HFHS + 250 mg/kg S and HFHS for PPARα gene expression was significant ($P = 0.030$).

Conclusion: The present study showed that consumption of saffron could prevent developing NAFLD in rats at least partially through modulation in gene expression of PPARα.

Keywords: Nonalcoholic Fatty Liver Disease, Saffron, Liver Enzymes, Blood Lipids, Liver Histopathology, Insulin Resistance, Inflammation, Gene Expression

Conflicts of Interest: *None declared*
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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the world (1). In Asian countries, NAFLD starts at ages between 12.5 and 17.5 (2). NAFLD is formed by the accumulation of fats, especially triglycerides, more than 5% of the weight of the liver itself (3). The mechanisms for this disease are not known very well but could be caused by insulin resistance and reduction in utilization of fatty acids at the cellular level as a result of mitochondrial malfunction or lower production of VLDL (4). To date, no definitive treatment has been found for NAFLD (2, 5). In patients with high-fat penetration, a rapid weight reduction would exacerbate inflammation, fibrosis, and bile duct obstruction. Also, no medication has been found to reverse liver damage without weight reduction independently and these medicines have numerous side effects (5). Antioxidants by their abilities in preventing lipid peroxidation, play a pivotal role in preserving the qualities of foods (6). Antioxidants because of their ability to reduce oxidative

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↑What is "already known" in this topic:
Saffron is known as an anti-diabetic, hypolipidemic, antiobesity, and anticancer spice and probably is an effective intervention for the prevention of NAFLD.

→What this article adds:
Despite its widespread use in the treatment of metabolic diseases, to our knowledge, this is the first study that evaluated the effects of saffron in the prevention of NAFLD.

stress are important for health (7). Saffron is rich in carotenoids (Crocin), glycoside (Picrocrocin), and a volatile fatty substance (Safranal) (8). Recently, extensive studies have established this substance as a strong antioxidant (9-11) that protects tissues (12-14). Crocin extract was able to significantly ameliorate hyperlipidemia by inhibiting pancreatic lipase. In a recent study, it was shown that a 1% increase of crocin in diet resulted in protecting rats against induced liver damages by B1 aflatoxin and dimethyl-nitrosamine. Crocin induced these results by inhibition of enzymes like ALP, and LDH; however, an increase in the dose of crocin did not have general protection effects (15). In another study, a daily injection of 200 mg/kg crocin resulted in the protection of liver cells against induced damage of iron, resulting in meaningful improvements in liver function and reduction of blood urea (11). In general, saffron's protective mechanisms are not specifically identified but most likely its protective effects are related to oxidative paths (16). Several studies have reported that saffron can be useful as an antioxidant in the management of some chronic disorders, especially inflammatory diseases. Given the contradictory results and limited studies, we performed this study to evaluate the effects of saffron on biochemical analyses and FAS, ACC1, CPT1, PPARα, DGAT2, SREBP 1-c gene expression for finding the mechanical effects of saffron supplementation in preventing NAFLD in a rat model.

Methods

Animals and Diets

In this experimental study, 12 male Sprague–Dawley rats weighing 200 ± 10 g were purchased from the animal laboratory of the Razi Institution. Each rat was kept in a separate cage during the study. Then, animals were acclimatized in a standard environment at 22°C ± 2°C temperature, 50 % humidity, and 12-h light/dark cycles with ad libitum access to food and water for 1 week in the Experimental Studies Center of Iran University of Medical Science. During the first week, all animals were fed a standard laboratory chow diet (Behparvar); afterward, they were randomly divided into 2 groups to be evaluated for saffron prevention effects that was designed for 7 weeks. The animals were randomly assigned into 2 groups, either fed with a high-fat, homemade diet + 250mg/kg saffron (HFHS+ 250 mg/ kg S) or fed with a high-fat, high-sugar homemade diet (HFHS). The high-fat, high-sugar homemade diet was prepared by mixing the following items: 28% butter, 28% chow diet, 14% sugar, 19% yolk egg, and 11% white egg. This mixture was cooked in the oven at 100°C for 30 minutes and was cut into small cubes. It included 63% of energy derived from fat, 27% from carbohydrates, and 10% from protein (17). The diets were prepared weekly and stored as vacuum packed (500 g) at −20°C. Packs taken for use were thawed in the refrigerator at 4°C. The food was offered daily, the residual was weighed, and removed after 48 hours. Body weights in grams were recorded at the baseline and every 2 weeks. Also, food intakes were measured twice a week with a digital scale (Orbit, KS 02). At the end of the study, rats were anesthetized in the overnight fasting state by injecting ketamine hydrochloride (70mg/kg) and xylazine (7 mg/kg) for further investigation. Animal experiments were conducted in conformity with the National Institutes of Health's ethical guidelines for the care and use of laboratory animals (NIH; Publication No. 85-23, revised 1985) and were approved by the Veterinary Ethics Committee of Iran University of Medical Sciences with ethics code of 27-4-1394- 25433.

Saffron Powder

In our study, saffron was provided by Novin Saffron Company (Mashhad, Iran). According to the daily weight of the rats, 250 mg/kg saffron powder was added to the high-fat high sugar diet in the prevention phase.

Tissue and Blood Preparation

Blood samples were taken from the heart of the rats and were collected in heparinized tubes. Then, they were centrifuged (3000 rpm, 5 min, 6$^{\circ C}$) to obtain the serum. Samples were kept at –80$^{\circ C}$ before biochemical analysis. After collecting blood samples, parts of the liver were excised, washed with cold physiologic saline (0.9%), and dried. One lobe of each liver tissue was preserved in a 10% buffered-formalin solution for histopathologic examination.

Biochemical Assessments

Plasma concentrations of ALT, AST, GGT, ALP, and serum lipids, including TC, HDL, LDL, and TG were measured by applying Parsazmoon kits (Tehran, Iran) in Noor pathobiology laboratory (Tehran, Iran). Also, insulin concentrations were measured using a rat insulin radioimmunoassay kit at 4°C (Linco Research Inc). Plasma glucose was measured through colorimetric methods by using a commercial kit (Parsazmoon). Eliza kit specifically for the rat was used to determine high-sensitivity C-reactive protein (hs-CRP) (hs-CRP; Cusabio Biothech), total antioxidant capacity (TAC) was measured according to Koracevic et al, and HOMA-IR scores were calculated using fasting serum insulin and glucose concentrations at the end of the experimental period according to the following formula: (20. Insulin/glucose-3.5) %.

Histopathology

Five parts of the liver tissue were first submitted and processed through ethyl alcohol and xylene series and then embedded in paraffin to produce blocks for excision. Slides were stained with hematoxyline eosin and Masson trichome and viewed under light microscopy by Novex Lenz 40. The grading was defined as follows: for hepatic steatosis: grade 0 (normal), grade 1 (<33% accumulation of fat of the hepatic parenchyma), grade 2 (between 34%-66% accumulation of fat of the hepatic parenchyma), and grade 3 (>66% of the accumulation of fat of the hepatic parenchyma).

Gene Expression

RNA isolation, reverse transcription, and reverse transcription polymerase chain reaction (RT-PCR): Liver tissue from rats sacrificed at 24 hours after drug administration was dissected over cold PBS. Total RNA was extracted from tissue using a high RNA isolation kit (CAT No. 118286650011). The quantity and quality of the isolated

Table 1. Primers used for quantitative Real-Time PCR analysis

Gene	Type	Sequence	Gene ID
SREBP 1-c	Forward	5'- CTA CCG TTC TAT CAA TGA CAA G-3'	6720
	Reverse	5'- CTG GTT GCT GTG CTG TAA GAA G-3'	
PPARα	Forward	5'-CGC AGG AAA GAC TAG CAA CAA TC-3'	25747
	Reverse	5'-TGG CAG CAG TGG AAG AAT CG-3'	
FAS	Forward	5'-TCA GTC TCA TTT CCT CAG TCT TCC -3'	50671
	Reverse	5'- GAC GCC TCA GTT CAC AGT ATT ATG -3'	
CPT1	Forward	5'-AAG TCA ACG GCA GAG CAG AG -3'	25757
	Reverse	5'- ACA CCA CAT AGA GGC AGA AGA G-3'	
ACC1	Forward	5'-TGG CTG GAC AGA CTG ATT GC -3'	60581
	Reverse	5'-AGG AGT GAA AGT TAG AGA TGC -3'	
DGAT2	Forward	5'- AGT CCT ACA GTG GGT CCT ATC C-3'	252900
	Reverse	5'-GGC GTG TTC CAG TCA AAT GC -3'	

FAS: Fatty Acid Synthase; ACC1: Acetyl-CoA carboxylase; CPT1: carnitine palmitoyltransferase 1; PPARα: Peroxisome proliferator-activated receptor alpha; DGAT2: diacylglycerol acyl transferase 2; SREBP 1-c: Sterol-regulatory element-binding protein 1c.

RNA were determined by measurement OD of 260 and 280 nm using Ultrospec 2000 UV/VIS Spectrophotometer (Pharmacia) and agarose gel electrophoresis. cDNAs were generated from 1µg total RNA by reverse transcription using the Cycle Script Reverse Transcription system (Bioneer). The mRNA expression levels of AS ACC1, CPT1, PPARα, DGAT2, SREBP 1-c, and GAPDH were determined by quantitative real-time RT-PCR, using a rotor-gene 6000 real-time thermal cycler. GAPDH mRNA was used as an internal control. All primers were purchased from Qiagen (Table 1). The PCR reactions were set up in a volume of 10 µL, containing 1 µL cDNA, 5 µL AccuPower 2X GreenStar qPCR Master Mix (Bioneer), and 10 Pm of each forward and reverse specific primer. Reaction mode was set at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Amplification specificity was checked by verifying a single peak in melting curves. All samples and controls were normalized against the reference gene. No template controls and no reverse transcriptase control was included in the PCR run. All assays were performed 3 times as independent PCR runs for each cDNA sample. The ΔΔCT method (18) was used to quantify the amplification-fold difference between groups; each gene expression was normalized with respect to the GAPDH mRNA content.

To validate the use of the ΔΔCT method, a 5-fold serial dilution was performed on a cDNA sample over a 125-fold range. For each dilution sample, amplifications were performed in triplicate using reference and target gene primers. The average computer tomography (CT) of all tests was calculated and the ΔCT of a target (FAS,ACC1, CPT1,PPARα ,DGAT2, and SREBP 1-c) and reference (GAPDH) genes was determined. A plot of the log cDNA dilution versus ΔCT ($\Delta CT_{target} - \Delta CT_{reference}$) was made for each target and reference genes and the slope of the fitted line was determined (18).

Statistical Analysis

SPSS 22 (SPSS Inc) was used to perform the statistical analysis of the data. The Kolmogrove-Smirnov test was used to evaluate the normal distribution of the data in the prevention phase; then, the differences among groups were evaluated by the Mann-Whitney test for non-normal data and the independent t test for normal data in this phase. One-way analysis of variance test was used to evaluate the homogeneity of the data in the treatment phase; next, the differences among groups were evaluated by the post hoc (Scheffe) test for homogeneous data and the Kruskal-Wallis test for heterogeneous data. The Fisher test was also used to evaluate the degree of the disease. The data are demonstrated as means ± standard deviation and $P < 0.05$ was defined as significant.

Results

Effects of Saffron on NAFLD

To demonstrate the effects of saffron on NAFLD prevention, 12 rats were assigned into 2 groups, either in the HFHS or the HFHS+250 mg/kg S groups for 7 weeks.

Changes in Body Weight and Food Intake During the Prevention Phase

As indicated in Table 2, the baseline body weights were similar among the 2 groups ($P = 0.441$). There was a significant elevation in body weight of the HFHS group versus the HFHS + 250 mg/kg S group ($P = 0.034$) and a significant elevation in food intake of HFHS versus HFHS+250 mg/kg S group ($P = 0.001$).

Table 2. Changes in body weight and food intake of rats with high-fat, high sugar (HFHS) and high-fat, high sugar homemade diet + 250mg/kg saffron (HFHS+ S) diet in prevention phase

Variables	HFHS	HFHS+S	*P*
Initial weight (gr)	198.83±4.07	196.66±4.27	0.441
Terminal weight (gr)	291.50±41.61	259.33±24.84	0.034
Food intake (gr)	497±39.27	405.50±49.77	0.001

Data are presented as means ± SD. The *P*-value indicated intragroup differences compared to independent samples t test. $P < 0.05$ was considered statistically significant.

Table 3. Serum level of hepatic enzymes, serum lipids, glycemic profiles, antioxidant and inflammatory factors of rats with high fat, high sugar (HFHS) and high-fat, high sugar homemade diet + 250 mg/kg saffron (HFHS+ S) diet in the prevention phase

Variables	HFHS	HFHS+S	*P*
ALT (U/L)	62.83±5.49	41.60±5.17	0.011
AST (U/L)	144.00±85.81	108.50±18.09	0.010
GGT (IU/L)	1±00	1±00	0.242
ALP (IU/L)	580.33±105.97	702.16±204.61	0.220
TG (mg/dL)	42.16±9.45	52.33±10.02	0.040
Cholesterol (mg/dL)	70.16±8.97	77.83±10.25	0.841
HDL.c (mg/dL)	24.16±1.47	22.16±1.32	0.242
LDL.c (mg/dL)	12.66±4.32	14.50±5.82	0.741
FBS (mg/dl)	239.66±31.12	188.83±32.04	0.001
Insulin (µU/ml)	0.53±0.37	0.41±0.22	0.035
HOMA-IR	0.29±0.16	0.19±0.22	0.032
TAC	0.44±0.33	0.55±0.45	0.041
hs-CRP	2.72±1.85	1.31±1.30	0.934

Data are presented as means ± SD. The P-value indicated intragroup differences compared to the independent samples t test with normal distribution and the Mann-Whitney with non-normal distribution. $P < 0.05$ was considered statistically significant.

Table 4. Percent of the grade of NAFLD in the prevention phase

Variable	Group1 HFHS+250 mg/kg S[b] N=6	Group2 HFHS[c] N=6	*P* value[d]
Normal liver	46.7%	16.7%	
Grade 1	53.3%	50%	0.025
Grade 2	0%	0%	
Grade 3	0%	33.3%	

[a]Data are presented as mean ± SD. [b] High fat, high sugar diet+ 250 mg/kg saffron. [c]High fat, high sugar diet. [d]P < 0.05 was significantly based on the Fisher test.

Changes in Hepatic Enzymes, Serum Lipids, Glycemic, Inflammatory, and Antioxidant Factors

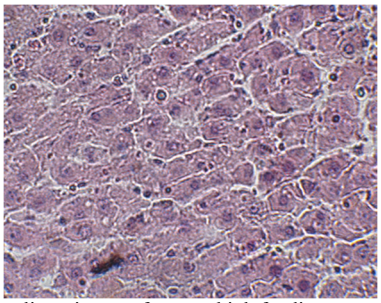
The data in Table 3 show that differences regarding the serum levels of liver enzymes, including GGT and ALP were not significant between groups 1 and 2 in the prevention phase ($P = 0.242$, $P = 0.220$) except for ALT ($P = 0.011$) and AST ($P = 0.010$). Also, the HFHS group had higher plasma levels of FBS ($P = 0.001$), insulin ($P = 0.035$), HOMA-IR ($P = 0.032$), and lower TAC ($P = 0.041$) versus the HFHS+ S group. There was not a marked increase in hs-CRP of the HFHS group versus HFHS+ S ($P = 0.934$). Plus, serum levels of lipids, such as TC, HDL, and LDL cholesterol, were not significant between groups 1 and 2 ($P = 0.841$; $P = 0.242$, $P = 0.741$) except for TG ($P = 0.040$) in the prevention phase.

Histological Observations

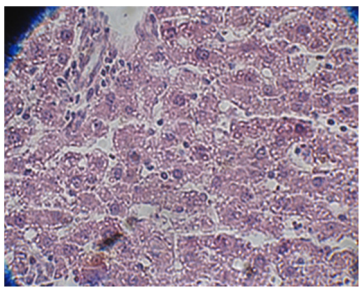
Table 4 reports the percent degree of the disease in the prevention phases. According to pathologic and microscopic tests on liver tissues of rats, 46.7 % of rats in the HFHS+ 250 mg/kg saffron group had normal liver and 53.3% of them had NAFLD with grade 1, but 16.7% of the rats in the HFHS group had normal liver and 50% of them had NAFLD with grade 1, and 33.3% of rats in this group had NAFLD with grade 3. Thus, this difference was statistically significant ($P = 0.025$) in the prevention phase. Moreover, the effects of saffron powder on liver steatosis based on histopathological examination (hematoxylin and eosin [H&E] staining) was observed in the prevention group (Figure 1). Major histopathological changes induced by HFD in rat liver were hepatosteatosis, ballooning, and inflammation of hepatocytes.

Gene Expression

Figure 2 demonstrated that in the prevention phase, PPARα gene expression showed a significant difference between groups. However, SREBP-1c, DGAT2, FAS,



a. liver tissues of rats on high fat diet



b. liver tissues of rats on high fat diet plus saffron 250mg/kg

Figure 1. Effects of saffron powder on liver steatosis based on histopathological examination (hematoxylin and eosin [H&E] staining) in the prevention group. Representative histopathological examination of H&E staining of liver tissue prepared from experimental rats fed with (a) high-fat diet (HFD), (b) high-fat diet + Saffron powder 125 mg/kg (HFD + L-CRO), (magnification 40×). Major histopathological changes induced by HFD in rat liver were hepatosteatosis, ballooning, and inflammation of hepatocytes.

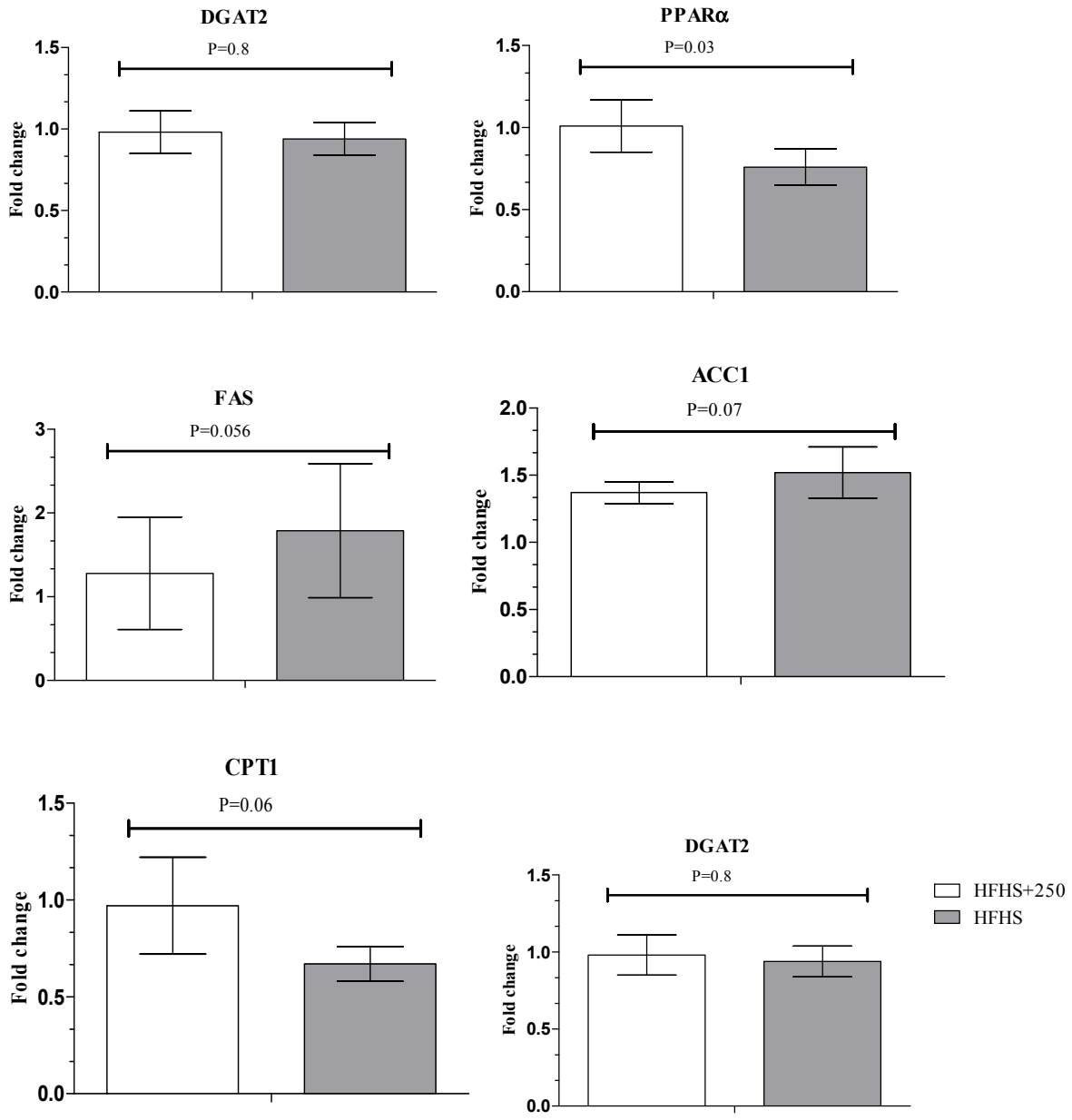


Figure 2. Gene expression of SREBP 1-c, FAS, ACC1, CPT1, PPARα, DGAT2 in rat liver tissue at the prevention phase. Data are presented as mean ± SD. $P < 0.05$ is significant based on the t test and the Mann-Whitney test.

ACC1, and CPT1 were not significantly different between the 2 groups.

Discussion

To our knowledge, this is the first study that evaluated the effects of saffron in the prevention of NAFLD in an animal model. NAFLD is a progressive disease that can progress toward fatal destruction of the liver and eventually death (19). NAFLD is relevant to obesity, diabetes, hyperlipidemia, insulin resistance, and high blood pressure, which are recognized as the main factors in metabolic syndrome (20, 21). Although polyphenols are types of the suggested nutraceuticals as the promising antioxidants to heal NAFLD and diminish fat assembly, there is still little knowledge about the key mechanisms involved in the inception and development of NAFLD considered as the liver element of the metabolic syndrome (22). Saffron belongs to the Ivadacae group and is widely cultivated in Iran, Spain, Greece, and India. For more than 2 decades, saffron has been used widely as a spice with known therapeutic benefits (23) and is used as a strong curative medicine supported by extensive medical studies (24).

Our results revealed that saffron consumption showed a significant effect on reducing serum TG ($P = 0.04$) in rats that were fed with 250 mg/kg saffron in the prevention phase. however, other lipid profiles (HDL, LDL, TC) did not show a significant difference in the prevention phase. Crocin capsule (15 mg/kg for 8 weeks) has been reported

not to have significant results in normalizing lipid profiles of patients affected by metabolic syndrome (25). In addition, it has been reported that saffron was not effective in reducing blood lipids after 12 weeks in patients suffering from schizophrenia and metabolic syndrome (26). Another study reported that saffron-laced tea (1 g saffron added to the tea for 8 weeks) had a significant impact in stabilizing TC, LDL, and HDL in patients suffering from type 2 diabetes (27). In a study on diabetic rats affected by encephalopathy, saffron extract in 40 and 80 mg/kg doses demonstrated significant reductions in TC, LDL, and TG and a significant increase in HDL after 28 days (28). An 8-week study on rats also reported that crocin extract in 80 mg/kg dose resulted in a reduction of total body fat, TC, and TG, while saffron extract in 40 mg/kg dose resulted in the reduction of LDL/HDL ratio (29). Crocin extract in 25, 50, and 100 mg/kg doses in rats fed with a high-fat diet for 10 days has been also reported to significantly reduce TG, TC, LDL, and VLDL via inhibiting pancreatic lipase as a key enzyme in triglyceride metabolism (30). Therefore, saffron seemed to have played the role of an efficient inhibitor to reduce TG in our study.

The results of the present study showed that saffron had no significant role to normalize hepatic enzymes, including GGT and ALP, in the prevention phase, but it could meaningfully diminish ALT and AST in rats that were fed with 250 mg/kg saffron in the prevention ($P = 0.01$) phase. It can be stated that saffron seems to protect the membrane of hepatocytes strongly due to its polyphenols and antioxidants that can lead to controlling and managing the levels of enzymes inside the cells. The differences in our results compared with the mentioned studies might be either due to the baseline healthy status of our rats or it might have been due to variable doses of saffron in prevention phases.

In this study, it was also indicated that saffron has meaningful reduction effects on weight and food intake in the prevention phase. Our results are consistent with a study that reported that ethanolic extract of saffron (176.5 mg/d for 2 months) led to a significant weight loss in overweight women (31). There are scattered articles that studied the nutritional mechanism of saffron in metabolic diseases about weight loss or controlling appetite. However, being stated as an antioxidant or an agent to increase antioxidant enzyme gene expression because of its abundant polyphenols and carotenoids (32), saffron seems to suppress appetite and reduce food intake via increasing serotonin in the central nervous system and digestive system (23).

In the present study, it was expressed that saffron could prevent developing NAFLD to severe forms in the prevention phase. We can assume that the use of saffron simultaneously with the high- fat, high-sugar diet did not allow the TG accumulation occur in hepatocytes in the prevention phase.

The results of the present study in the prevention phase showed significant changes in serum levels of blood glucose, insulin, and insulin resistance. Our results were inconsistent with those of Nikbakht et al study and consistent with those of Liang Xi et al and Milajerdi et al studies (33-35). Liang Xi et al conducted 40 mg/kg saffron extract in rats for 8 weeks and concluded that saffron significantly increases the insulin resistance and the expression of the adiponectin gene via regulation of FFA metabolism in white fat tissue. Nikbakht et al conducted 15 mg/kg S extract and their results showed no significant effects of saffron on blood glucose. It seems that our short period of intervention is the possible reason for this inconsistency (25). The main reason for inconsistency between the findings may be the result of the dose of saffron that was used in our study or the short duration of our study that could also be other causes of not observing consistent results with the study of Nikbakht et al.

In the prevention phase, our findings showed no significant changes in serum levels of hs-CRP. To our knowledge, the present study was the first to investigate the effect of saffron on serum levels of hs-CRP and there is no other study to compare with our results.

De novo fatty acid synthesis occurs via a complex series of reactions that take place in the mitochondrial matrix and the cytosol of hepatocytes. The rate of de novo lipogenesis is dependent on the transport activity of the mitochondrial CiC and enzymatic activities of ACC, FAS, DGAT 1 and 2, and SCD1. Induction of lipogenic genes occurs in response to the combined actions of several nuclear transcription factors: SREBPs, ChREBP, LXRα, FXR, and PPARs (36). In the prevention phase, PPARα decreased and showed a significant difference between groups, and ROS production also had an indirect hepatotoxic effect via mitochondrial β-oxidation. Fatty acids are transported into the mitochondrial matrix by a carnitine-dependent enzyme shuttle. Mitochondrial β-oxidation implies a series of dehydrogenation, hydration, and cleavage reactions catalyzed by membrane-bound and soluble enzymes transcriptionally regulated by PPARα (37). FFR-induced PPAR-α up-regulates CPT-1 expression and increases the mitochondrial β-oxidation, and regulates the uptake and clearance of fatty acids. Thus, the PPARs regulate fatty acid metabolism and storage. Knockout models of PPAR-α have been associated with steatosis, implicating a possible role for PPAR-α in NAFLD (37). In this study, it was expressed that saffron could help prevent the developing NAFLD to severe forms in the prevention phase. We can assume that the use of saffron simultaneously with the high-fat, high- sugar diet did not allow the TG accumulation occur in hepatocytes in the prevention phase. Thus, consumption of saffron in this study could partly prevent development NAFLD in rats and its mechanism of effect may be due to effect on PPARα.

Our study's strength was using saffron powder rather than tablets or extract since it was more convenient and maintained more of its vitamin and polyphenol content over time. We designed a rat model of nonalcoholic fatty liver disease using dietary components, including sugar, egg yolk, and animal fat that this dietary pattern can be used by researchers to design an animal model of NAFLD for cost management. The limitation of our study was the short period of intervention and a small number of rats.

Conclusion

In conclusion, saffron could be a promising source to protect the liver against developing NAFLD, normalizing liver

enzymes, lipid profiles, and insulin resistance because of its strong polyphenols that may confirm our hypothesis. Since it was the first study to investigate the histopathology of nonalcoholic fatty liver in a rat model of the prevention phase, we could not compare this result with previous similar studies. Hence, further in vitro or in vivo studies should be performed to determine the metabolic mechanism of saffron to find its significant effect on the improvement of liver histopathology, lipid profiles, and hepatic enzymes. To learn more about the key mechanisms of saffron involved in liver function, these investigations are advised to be conducted using both animal and human studies, including providing the appropriate dose, extending the inquiry period, or even increasing the sample size.

Acknowledgment

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Authors Contribution

N. Aryaeian and R. Alipour: study conception and design. M. Barati and N. Aryaeian: data acquisition. M. Soleimani and N. Aryaeian: analysis and interpretation of data. G. Hajiluian: drafting of the manuscript. N. Aryaeian: critical revision.

Abbreviations

NAFLD, nonalcoholic fatty liver disease
HFHS, high-fat, high-sugar diet
HFHS+ 250 mg/ kg S, high-fat, high-sugar diet + 250 mg/kg saffron
C, Chow diet
ACC1, acetyl-CoA carboxylase 1
CPT1, carnitine palmitoyltransferase I
PPARα, peroxisome proliferator-activated receptor α
DGAT2, diacylglycerol O-Acyltransferase 2
SREBP 1-c, sterol regulatory element-binding protein-1c
VLDL, Very low-density lipoprotein
STZ, streptozotocin
ALP, alkaline phosphatase
LDH, lactate dehydrogenase
HFD, high-fat diet
ALT, alanine aminotransferase
AST, aspartate aminotransferase
RNA, ribonucleic acid
RT-PCR, real time-polymerase chain reaction
PBS, phosphate-buffered saline
cDNA, complementary deoxyribonucleic acid
GAPDH, glyceraldehyde-3-phosphate dehydrogenase
CiC, citrate carrier
SCD1, critical role of stearoyl-CoA desaturase–1
ChREBP, carbohydrate responsive element binding protein
LXRα, liver X receptor α
FXR, farnesoid X receptor
ROS, reactive oxygen species
FFA, free fatty acids
TG, triglyceride
TA, total cholesterol
LDL, low-density lipoprotein
HDL, high-density lipoprotein
PPARα, peroxisome proliferator-activated receptors
GAPDH, glyceraldehyde-3-phosphate dehydrogenaseRT-RCR, Reverse transcription polymerase chain reaction
FBS, Fetal bovine serum

Ethical Issue

The Ethics Committee of Iran University of Medical Sciences approved the study protocol (approval ID: 27-4-1394- 25433).

Conflict of Interests

The authors declare that they have no competing interests.

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