Research Article

Saffron has a therapeutic effect on nephropathy by regulating the expression of TLR4, S100A8, and HMGB1 genes and reducing oxidative stress in diabetic rats

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ABSTRACT

Objectives: Diabetic nephropathy (DN) is a major complication of diabetes that requires effective treatment options. This study explores the potential benefits of saffron extract as a remedy derived from medicinal plants, focusing on its effects on key inflammatory genes—Toll-like receptor 4 (TLR-4), S100 calcium-binding protein A8 (S100A8), and High Mobility Group Box 1 (HMGB1)—as well as its role in reducing oxidative stress in the kidney tissue of rats with type 1 diabetes.

Methods: The rats were randomly divided into 8 groups of 6 each. Diabetes was induced using streptozotocin (55 mg/kg.bw). Diabetic and control groups were treated with three doses of saffron extract (100, 200, and 300 mg/kg) for 60 days. Biochemical kits were used to evaluate fasting blood glucose (FBG), urea, creatinine, albumin, lipid profile, malondialdehyde (MDA), and total antioxidant capacity (TAC). Expression of TLR-4, S100A8, and HMGB1 genes were evaluated by real-time PCR. ANOVA and Bonferroni post-hoc tests were used for data evaluation.

Results: Diabetes significantly impaired the FBG, lipid profile, creatinine, urea, and albumin levels ($P<0.05$). After treatment with the saffron extract, these parameters were significantly close to the normal range in all groups compared to the control group ($P<0.05$). Also, the saffron extract significantly decreased the expression levels of TLR-4, S100A8, and HMGB1 genes and improved oxidative stress markers (TAC and MDA) in kidney tissues when compared to the diabetic control group ($P<0.05$). In addition, the beneficial effects of saffron were dose-dependent.

Conclusion: Based on the obtained results, saffron extract can lead to the improvement of nephropathy by reducing the expression of TLR-4, S100A8, and HMGB1 genes as well as improving oxidative stress. Thus, it may be used as an adjuvant treatment for diabetic complications.

Keywords: Saffron, Hydroalcoholic Extract, Oxidative stress, Gene Expression, Diabetes, rat

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Introduction

Diabetic nephropathy (DN) is one of the most common and serious complications of diabetes mellitus, affecting approximately 30-40% of individuals with diabetes over the course of their disease (1). Its prevalence is particularly high among patients with poorly controlled blood glucose levels and those with a long duration of diabetes (2). The pathogenesis of DN is multifactorial, involving a complex interplay of metabolic and hemodynamic factors, including hyperglycemia, increased production of advanced glycation end products (AGEs), and activation of the renin-angiotensin-aldosterone system (RAAS) (3). These processes lead to renal hypertrophy, glomerular hyperfiltration, and ultimately glomerulosclerosis and tubulointerstitial fibrosis. Complications of DN include progressive renal impairment, which can lead to end-stage renal disease (ESRD), cardiovascular disease, and increased morbidity and mortality (4, 5). Current treatment strategies focus on glycemic control, blood pressure management, and the use of renin-angiotensin system inhibitors, which have been shown to slow the progression of renal disease (6). However, the need for more effective therapeutic options remains critical, as DN continues to pose significant challenges in the management of diabetes-related complications.

The pathogenesis of DN involves complex interactions between metabolic dysregulation and inflammatory processes, with emerging evidence highlighting the roles of various molecular mediators (7). Toll-like receptor 4 (TLR-4), a key player in the innate immune response, has been implicated in the amplification of inflammatory signaling in DN (8). Concurrently, S100A8, a member of the S100 protein family, acts as a pro-inflammatory mediator, promoting renal inflammation and fibrosis (9). High Mobility Group Box 1 (HMGB1), a well-known damage-associated molecular pattern (DAMP), is released during cellular stress and injury, further exacerbating inflammation and contributing to renal damage (10).

The management of diabetes and its complications, particularly diabetic nephropathy, has increasingly turned to natural remedies and medicinal plants, with saffron (Crocus sativus) gaining attention for its potential therapeutic effects (11). Saffron contains bioactive compounds, such as crocins and safranal, which exhibit anti-inflammatory, antioxidant, and anti-diabetic properties (12). Research suggests that saffron may help regulate blood glucose levels, improve insulin sensitivity, and reduce oxidative stress, all of which are critical in the management of diabetes (11). Additionally, saffron's nephroprotective effects have been demonstrated in various studies, indicating its ability to mitigate renal damage and inflammation associated with diabetic nephropathy (13).

In light of the growing evidence supporting the therapeutic potential of saffron in managing diabetic complications, this study aims to investigate the specific mechanisms by which saffron exerts its protective effects on diabetic nephropathy. By focusing on the regulation of key inflammatory mediators such as TLR4, S100A8, and HMGB1, as well as the modulation of oxidative stress pathways, we seek to elucidate the underlying molecular mechanisms that contribute to the nephroprotective properties of saffron. Ultimately, this research aims to provide a foundation for the development of saffron as a complementary therapeutic agent in the treatment of diabetic nephropathy, potentially enhancing the current clinical strategies employed to combat this debilitating condition.

Materials and Methods

In this experimental study, 48 male rats were selected by simple random sampling from the Animal Breeding Center of the Arak University of Medical Sciences, Arak, Iran. Rats were kept in a temperature range of 23°C for 12 hours in the light and 12 hours in the dark, with free access to water and food. This study was conducted in compliance with all ethical codes for working with laboratory animals approved by the Ministry of Health and Medical Education, Iran.

Sample size

The sample size was calculated through G*Power 3.1 software based on previous studies, considering a power of 90%, an effect size of 0.55, and a significance level of 0.05 (14). The maximum sample size calculated by the software was six animals; therefore, for the 8 groups in this study, a sample size of six animals in each group was considered.

Preparation of hydroalcoholic extract of saffron

Dried saffron was purchased from the Barij Essence Pharmaceutical Company, Iran. The dried saffron was pulverized using an electric mill. Seventy percent ethanol was used to prepare the hydroalcoholic extract of the saffron. The resulting solution was mixed for 24 hours using a stirrer and filtered. The remaining precipitate was extracted thrice at room temperature using an ethanol hydroalcoholic solvent. The solution was concentrated under a vacuum using a rotary apparatus (Hidolf, Germany). To prepare the dry powder, the material was placed in an oven at 37°C for three days. Until testing, the extract was kept at -20°C in a freezer.

Induction of diabetes, experimental design and sample collection

Type 1 diabetes was induced in rats using a single

injection of streptozotocin (55 mg/kg.bw). To confirm the animals' diabetes three days following the STZ treatment, rats with a FBG level greater than 200 mg/dl were considered diabetic. After the induction of diabetes, the rats were divided into eight groups (each group included six animals) as follows:
Control group (C): This group received normal saline and a single dose of citrate buffer (0.1 M, pH 4.5). Control group of saffron extract (CS): This group received saffron extract in doses of 100 (CS100), 200 (CS200) and 300 (CS300) mg/kg.bw daily. Diabetic group (D): Diabetic control rats receiving normal saline. Diabetic group of saffron extract (DS): This group received saffron extract in doses of 100 (DS100), 200 (DS200) and 300 (DS300) mg/kg.bw daily.
The treatment was administered by oral gavage for 60 days. After 24 hours from the last treatment, the rats were anesthetized using ketamine and xylazine. Blood samples were collected via cardiac puncture, and the serum was quickly separated. Serum samples were aliquoted and stored at -80°C. Kidney tissue samples were removed, immediately frozen in liquid nitrogen, and stored at -80°C for RNA extraction.

Analysis of biochemical and antioxidant parameters

Enzyme-based commercial kits (Pars Azmoon, Tehran, Iran) were used according to the manufacturer's instructions with a spectrophotometer (Jenway 6505, European Union) to measure serum levels of FBG, urea, creatinine, albumin, and lipid profile [triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL)]. The serum level of low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald Formula (15) as follows: LDL-c = Total cholesterol – [HDL-c + [TG/5]]. The total antioxidant capacity (TAC) and malondialdehyde (MDA) levels were measured in the kidney tissue according to the kit manufacturer's instructions (Teb Pazhouhan Razi (TPR), Tehran, Iran).

Analysis of S100A8, TLR4, and HMGB-1 gene expression in kidney tissue

The tissues of all rats were removed from the freezer at −80°C, then RNA was extracted from 50 mg kidney tissue using a column RNA isolation kit (Denazist's Kits Company, Mashhad, Iran), according to the manufacturer's recommendations. The 260/280 ratio was used to determine RNA purity and concentration. Complementary DNA (cDNA) was produced using 2 µg of intact RNA and oligo-dT primers (cDNA synthesis kit; Denazist's Kits Company, Mashhad, Iran). A 20-µL final volume was used for each cycle of quantitative real-time PCR (qRT-PCR): 10 µL SYBR Green qPCR MasterMix, 8 µL RNase-Free water, 0.5 µM of each primer, and 1 µL of cDNA. These studies were performed using the LightCycler 96 system (Roche Diagnostics GmbH, Mannheim, Germany). β-actin was used as housekeeping genes. The comparative Ct (2-ΔΔCt) method was used to evaluate data. The primer sequences were as follows: S100A8, 5′-AAATCACCATGCCCTCTACAAG-3′ (forward) and 5′-CCCACTTTTATCACCATCGCAA-3′ (reverse); TLR4, 5′-ATGGCATGGCTTACACCACC-3′ (forward) and 5′-GAGGCCAATTTTGTCTCCACA-3′ (reverse); HMGB-1, 5′-GCTGACAAGGCTCGTTATGAA-3′ (forward) and 5′-CCTTTGATTTTGGGGCGGTA-3′ (reverse); β-actin, 5′-GAGAAGATTTGGCACCACAC-3′ (forward) and 5′-CATCACAATGCCAGTGGTAC-3′ (reverse).

Statistical Analysis

The statistical significance of the data was estimated using one-way analysis of variance (ANOVA) and the Bonferroni post-hoc test. The experimental data are presented as the mean and Standard Error of the Mean (mean ± SEM). Statistical significance was set at $p < 0.05$. GraphPad Prism software, version 8, was used to statistically analyze the data.

Results

Results of FBG in the experimental groups

FBG levels were monitored at the beginning and end of the study (Table 1). Diabetes caused a significant increase in FBG, and administration of saffron for 60 days at doses of 100, 200, and 300 mg/kg.bw improved FBG ($P<0.001$) in a dose-dependent manner ($P<0.01$).

Results of lipid profile in the experimental groups

As shown in Table 2, the lipid profile was significantly disordered in the D group compared to the C group ($P<0.001$). The results also showed that administration of saffron for 60 days at doses of 100, 200, and 300 mg/kg.bw significantly improved the lipid profile ($P<0.001$). As reported, the effects of saffron on improving the lipid profile were dose-dependent ($P<0.001$).

Results of serum urea, creatinine, and albumin in the experimental groups

The levels of serum urea and creatinine in group D were significantly higher than those in group C ($P<0.001$) (Table 3). Serum urea and creatinine levels (100 mg/kg.bw, 200 mg/kg.bw, and 300 mg/kg.bw: $P<0.001$) were significantly reduced after administering saffron compared to the D group. In addition, the serum albumin level in group D was significantly lower than that in the C group ($P<0.001$). This disorder significantly improved after the administration of saffron compared to that in

Table 1. Effects of saffron on FBG concentrations of experimental groups.

Group	FBG, mg/dl	
	Zero day	60th day
C	86.8±5.7	75.5 ± 13.7
CS100	90.8 ±4.6	89.33 ± 3.4
CS200	91.5 ± 6.5	96.0 ± 5.5
CS300	88.33± 4.6	77.50 ± 4.6
D	416.5 ± 12.9	479.3 ± 32.3$^{a\ \dagger}$
DS100	394.2±7.2	246.3± 20.37$^{b\ \dagger}$
DS200	380.7 ± 6.4	165.3±5.4$^{b\ \dagger, c\ \#}$
DS300	388.5 ± 12.5	127.0±7.4$^{b\ \dagger, c\ \#, d\ ¥}$

Data are expressed as mean ± SEM; for all groups n=6; FBG: fasting blood glucose; C: Normal rats without receiving saffron; CS100: Normal rats treated with 100 mg/kg.bw saffron; CS200: Normal rats treated with 200 mg/kg.bw saffron; CS300: Normal rats treated with 300 mg/kg.bw saffron; D: Diabetic rats without receiving saffron; DS100: Diabetic rats treated with 100 mg/kg.bw saffron; DS200: Diabetic rats treated with 200 mg/kg.bw saffron; DS300: Diabetic rats treated with 300 mg/kg.bw saffron. [a] Compared with the C group; [b] Compared with the D group; [c] Compared with the DS100 group; [d] Compared with the DS100 and DS200 group; p-value † <0.001; p-value # < 0.01; p-value ¥ < 0.05. ANOVA and Bonferroni post hoc test analysis were applied to evaluate the data.

Table 2. Effects of saffron on lipid profile concentrations in experimental groups.

Group	TG, mg/dl	TC, mg/dl	LDL-c, mg/dl	HDL-c, mg/dl
C	67.0±5.2	88.33±4.9	31.33±3.8	40.8±5.2
CS100	72.5±11.7	79.33±9.3	23.5±3.2	41.5±6.2
CS200	73.0±4.8	91.67±4.0	22.0 ±2.1	38.67±2.6
CS300	66.1±8.4	89.17±7.9	23.33±1.8	45.67±6.6
D	147.0±7.3$^{a\ \dagger}$	202.7±16.5$^{a\ \dagger}$	99.33±4.7$^{a\ \dagger}$	16.33±2.5$^{a\ \#}$
DS100	111.2 ±4.9$^{b\ *}$	154.7±10.9$^{b\ *}$	73.33±2.6$^{b\ \#}$	38.17±3.9$^{b\ *}$
DS200	78.3±4.1$^{b\ \dagger, c\ *}$	107.7±5.0$^{b\ \dagger, c\ *}$	72.1±6.9$^{b\ \dagger, c\ ¥}$	41.67±6.2$^{b\ *, c\ *}$
DS300	45.5±3.3$^{b\ \dagger, c\ \dagger, d\ *}$	62.50±7.8$^{b\ \dagger, c\ \dagger, d\ *}$	48.3±4.5$^{b\ \dagger, c\ \#, d\ \#}$	48.0±2.9$^{b\ \#, c\ *, d\ *}$

Data are expressed as mean ± SEM; for each group n=6; TG: triglycerides; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; C: Normal rats without receiving saffron; CS100: Normal rats treated with 100 mg/kg.bw saffron; CS200: Normal rats treated with 200 mg/kg.bw saffron; CS300: Normal rats treated with 300 mg/kg.bw saffron; D: Diabetic rats without receiving saffron; DS100: Diabetic rats treated with 100 mg/kg.bw saffron; DS200: Diabetic rats treated with 200 mg/kg.bw saffron; DS300: Diabetic rats treated with 300 mg/kg.bw saffron. [a] Compared with the C group; [b] Compared with the D group; [c] Compared with the DS100 group; [d] Compared with the DS100 and DS200 group; p-value † <0.001; p-value # < 0.01; p-value * < 0.05;. ANOVA and Bonferroni post hoc test analysis were applied to evaluate the data.

Table 3. Effects of saffron on urea, creatinine, and albumin of urine and serum in experimental groups.

Group	Serum		
	Urea (mg/dl)	Creatinine (mg/dl)	Albumin(g/dl)
C	21.01±1.7	0.61±0.06	5.9±0.4
CS100	21.00±1.3	0.62±0.11	5.3±0.5
CS200	22.10±1.4	0.65±0.09	4.9±0.8
CS300	24.50±2.0	0.62±0.04	5.8±0.7
D	67.33±6.8$^{a\ \dagger}$	2.05 ±0.31$^{a\ \dagger}$	0.68±0.2$^{a\ \dagger}$
DS100	46.67±6.1$^{b\ \#}$	1.22±0.16$^{b\ \#}$	4.1±0.5$^{b\ *}$
DS200	27.83±1.7$^{b\ \dagger, c\ *}$	1.12±0.12$^{b\ \#, c\ ¥}$	4.7±0.4$^{b\ \#, c\ *}$
DS300	23.67±2.0$^{b\ \dagger, c\ \#, d\ *}$	0.70±0.06$^{b\ \dagger, c\ *\ d\ *}$	6.5±0.9$^{b\ \dagger, c\ *, d\ *}$

Data are expressed as mean ± SEM; for each group n=6. C: Normal rats without receiving saffron; CS100: Normal rats treated with 100 mg/kg.bw saffron; CS200: Normal rats treated with 200 mg/kg.bw saffron; CS300: Normal rats treated with 300 mg/kg.bw saffron; D: Diabetic rats without receiving saffron; DS100: Diabetic rats treated with 100 mg/kg.bw saffron; DS200: Diabetic rats treated with 200 mg/kg.bw saffron; DS300: Diabetic rats treated with 300 mg/kg.bw saffron. [a] Compared with the C group; [b] Compared with the D group; [c] Compared with the DS100 group; [d] Compared with the DS100 and DS200 group; p-value † <0.001; p-value # < 0.01; p-value * < 0.05; p-value ¥ > 0.05. ANOVA and Bonferroni post hoc test analysis were applied to evaluate the data.

the C group (100 mg/kg.bw: P<0.01, 200 mg/kg.bw: P<0.001, and 300 mg/kg.bw: P<0.001). It should be noted that the effects of saffron on serum urea, creatinine, and albumin levels were dose-dependent (P<0.05).

Results of the MDA and TAC levels in the kidney tissue of the experimental groups

The MDA and TAC levels in the kidney tissues of the experimental groups are shown in Table 4. The MDA level was significantly higher in the D group than in the C group (P<0.001), and TAC was significantly lower in the D group than in the C group (P<0.001). In addition, saffron administration at three doses caused a significant reduction in the level of MDA (100 mg/kg.bw: P<0.01, 200 and 300 mg/kg.bw: P<0.001) and a significant elevation in TAC (100 mg/kg.bw: P<0.01, 200 and 300 mg/kg.bw: P<0.001) in the kidney tissue of diabetic rats.

Table 4. Effects of saffron on MDA and TAC levels of kidney tissue in experimental rats

Group	MDA, nmol/mg protien	TAC, µmol/L
C	2.1 ± 0.04	292.3±25.1
CS100	2.2 ± 0.10	286.5±16.07
CS200	1.8 ± 0.08	279.3±11.73
CS300	1.9 ± 0.11	258.5±11.73
D	4.9 ± 0.15 [a†]	117.8±5.6 [a†]
DS100	3.8 ± 0.16 [b†]	165.8±7.59 [b¥]
DS200	3.1 ± 0.20 [b†, c¥]	264.5±10.35 [b†, c†]
DS300	1.7 ± 0.06 [b†, c†, d#]	318.2±20.78 [b†, c†, d¥]

Data are expressed as mean ± SEM; for each group n=6.MDA: malondialdehyde; TAC: Total antioxidant capacity. C: Normal rats without receiving saffron; CS100: Normal rats treated with 100 mg/kg.bw saffron; CS200: Normal rats treated with 200 mg/kg.bw saffron; CS300: Normal rats treated with 300 mg/kg.bw saffron; Dibt: Diabetic rats without receiving saffron; DS100: Diabetic rats treated with 100 mg/kg.bw saffron; DS200: Diabetic rats treated with 200 mg/kg.bw saffron; DS300: Diabetic rats treated with 300 mg/kg.bw saffron. [a] Compared with the C group; [b] Compared with the D group; [c] Compared with the DS100 group; [d] Compared with the DS100 and DS200 group; p-value † <0.001; p-value # < 0.01; p-value * < 0.05; p-value ¥ > 0.05. ANOVA and Bonferroni post hoc test analysis were applied to evaluate the data.

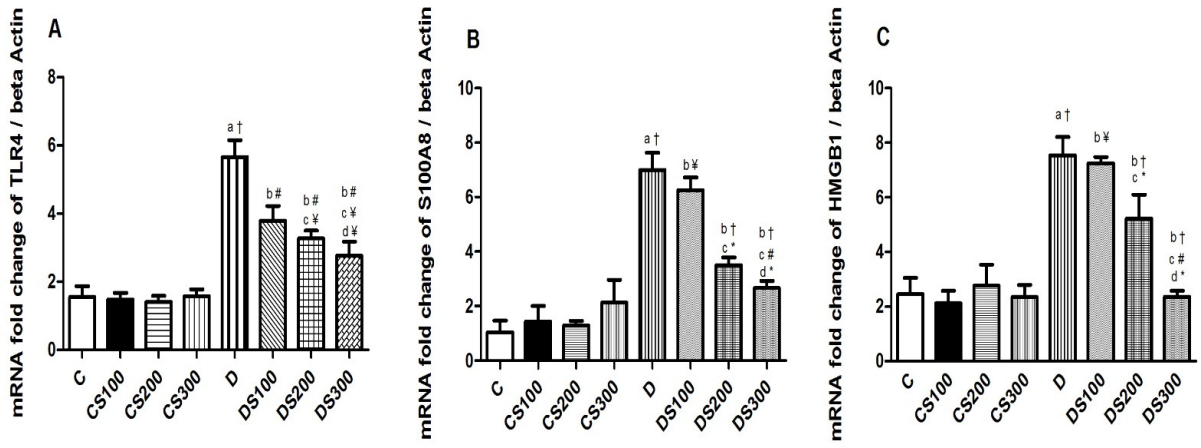

Figure 1-A, B & C: The effects of the saffron extract on TLR4 [A], S100A8 [B], and HMGB1 [C] mRNA folding change of kidney tissues in experimental groups. Data are expressed as mean ± SEM; for each group n=6. C: Normal rats without receiving saffron; CS100: Normal rats treated with 100 mg/kg.bw saffron; CS200: Normal rats treated with 200 mg/kg.bw saffron; CS300: Normal rats treated with 300 mg/kg.bw saffron; Dibt: Diabetic rats without receiving saffron; DS100: Diabetic rats treated with 100 mg/kg.bw saffron; DS200: Diabetic rats treated with 200 mg/kg.bw saffron; DS300: Diabetic rats treated with 300 mg/kg.bw saffron. [a] Compared with the C group; [b] Compared with the D group; [c] Compared with the DS100 group; [d] Compared with the DS100 and DS200 group; p-value † <0.001; p-value # < 0.01; p-value * < 0.05; p-value ¥ > 0.05. ANOVA and Bonferroni post hoc test analysis were applied to evaluate the data.

The effects of saffron on the levels of MDA and TAC were dose-dependent (P<0.05).

Results of the S100A8, TLR4, and HMGB-1 genes expression in the kidney tissue of the experimental groups

The results of the real-time PCR assay (Figure 1) showed that the gene expression of S100A8, TLR-4, and HMGB-1 in the kidney tissue of the D group was notably higher than in the C group (P<0.001). S100A8 (100 mg/kg.bw: P<0.01, 200 and 300 mg/kg.bw: P<0.001), TLR-4 (100 mg/kg.bw: P<0.01, 200 and 300 mg/kg.bw: P<0.001), and HMGB-1 (100 mg/kg.bw: P<0.01, 200 and 300 mg/kg.bw: P<0.001) gene expression was decreased significantly in the DS100, DS200, and DS300 groups after treatment with saffron compared to the D group. In addition, the effect of saffron on S100A8 and HMGB-1 gene expression in the kidney tissue of diabetic rats was dose-dependent (P<0.05).

Discussion

Today, the use of medicinal plants for metabolic and inflammatory diseases is increasing. One of the important medicinal plants is saffron. Saffron has received attention due to its anti-inflammatory and anti-diabetic properties. One of the serious complications of diabetes is nephropathy. Early diagnosis and treatment can prevent more serious kidney problems, such as chronic kidney disease (CKD). In this study, the anti-diabetic and nephropathy-improving effects of saffron were

investigated. Our results showed that DN significantly leads to an increase in the expression of TLR-4, S100A8, and HMGB1 and oxidative stress in the kidney tissue of diabetic rats, while the treatment with saffron improved them significantly in a dose-dependent manner.

The unique combination of crocins, picrocrocin, safranal, carotenoids, vitamins, minerals, flavonoids, and essential oils makes saffron a valuable spice with culinary, medicinal, and aromatic applications (16). Its constituents not only contribute to its flavor and aroma but also offer various health benefits, making saffron a subject of interest in both nutrition and pharmacology. It has been reported that the antidiabetic activity of saffron could be due to the presence of bioactive compounds such as crocin and β-carotenes (17, 18).

Induction of diabetes using STZ significantly increased FBG and lipid profile disorder, which is consistent with previous reports (19, 20). Our results showed that saffron treatment for 60 days can significantly reduce FBG and lipid profile in a dose-dependent manner, which is in line with the results of the study by Samarghandian et al. on the use of saffron to reduce FBG and improve the lipid profile (21). On the other hand, it has been demonstrated that crocin (one of the main compounds of saffron) has antidiabetic activity via an increase of GLUT4 translocation to the cell surface. Furthermore, it has been shown that saffron can also activate Akt kinase, stimulate phosphorylation of AMP-activated protein kinases (AMPK), acetyl-CoA carboxylase (ACC), and mitogen-activated protein kinases (MAPKs). In general, these effects show that saffron can lead to increased glucose uptake, reduced insulin resistance, and improved lipid profile (22, 23).

DN, due to damage to the kidney structure, leads to an increase in serum urea and creatinine and a decrease in serum albumin. Therefore, using these parameters to diagnose and monitor response to treatment can be useful (20). The results of our study showed an increase in serum urea and creatinine concentrations and a decrease in albumin concentration in the diabetic group compared with controls, which is in line with previous studies (24, 25). However, treatment with saffron in a dose-dependent manner significantly declined serum concentrations of urea and creatinine and increased albumin levels. Consistent with our results, Amri et al. reported that saffron can reduce serum urea and creatinine and increase serum albumin in diabetic rats (26). Also, Ebrahimi and Milajerdi and their colleagues showed in separate clinical trials that saffron can reduce serum urea and creatinine and improve nephropathy in type 2 diabetes patients (27, 28). It has been reported in several studies that serum concentrations of creatinine and urea of diabetic rats were significantly improved after treatment with crocin (24, 29). These observations were in line with our results.

Oxidative stress indicates that the balance between free radicals and their defense antioxidant system is disturbed (30). The increase in high blood glucose for a long time in diabetes leads to an increase in oxidative stress (31). According to the results of the present study, MDA levels in the kidney tissue of the diabetic group were significantly higher than in the control group. After saffron treatment, a significant decrease in MDA levels was observed. Saffron treatment also significantly increased TAC levels in the kidney tissue of the diabetic group. Evidence has shown that saffron and its compounds reduce lipid peroxidation in the tissues of rats exposed to oxidative stress (32, 33). Consistent with our study, Samarghandian's study revealed that saffron has antioxidant properties such as increasing glutathione, catalase, and superoxide dismutase activity while reducing malondialdehyde and nitric oxide in diabetic rats (34). It has been reported that crocin caused a significant decrease in MDA levels and a significant increase in TAC levels in the kidney and liver tissue of diabetic rats (25, 35). Overall, these results indicate that saffron, with its antioxidant properties, can be an adjunctive treatment for DN.

Studies have shown that expression disorders of genes involved in inflammation can contribute to the development of DN. Previous studies have reported that under diabetic conditions, the activity and expression of TLR4 in relevant kidney cells significantly increase and can ultimately lead to DN (36). Zhu T and colleagues showed that people with DN have high levels of TLR-4, TNF-α, and IL-6 (37). Also, Yang Liu et al. examined the role of TLR-4 in DN in adult male rats and found that diabetic mice had higher TLR-4 expression in the renal glomerulus, proximal gastrointestinal tract, and interstitial kidney regions (38). The results of our study also showed that the expression level of TLR-4 in the kidney tissue of diabetic rats increased, which was significantly reduced after treatment with saffron. In this regard, Poursamimi et al. reported that saffron, by reducing the expression of TLR-4 and IL-6 in the kidney tissue of rats with ischemic nephropathy, led to its improvement (39). These suppressive effects on TLR-4 expression are probably due to the presence of crocin, as various studies have reported that crocin has suppressive effects on TLR-4 expression (40, 41). S100A8 is a calcium-dependent protein that plays an important role in regulating inflammatory processes. Studies have shown that S100A8 can increase the production of inflammatory cytokines by binding to TLR-4 receptors. Du et al. reported that the expression of S100A8 and S100A9 in the kidney tissue of diabetic rats was significantly increased. They also reported that suppressing their expression with S100A8/A9 expression/knockdown lentivirus could ameliorate DN (42). The results of our study showed that saffron can significantly reduce S100A8. To our knowledge, the effect of saffron on S100A8 expression has not been reported. However, studies suggest that plant polyphenols such as resveratrol and curcumin can reduce S100A8 expression

in diseases such as rheumatoid arthritis and chronic myeloid leukemia, thereby reducing inflammation and leading to improvement in these diseases (43, 44). These results confirm the inflammatory effects of S100A8 and its potential as a target for treatment in inflammatory diseases such as DN. HMGB1 is a nuclear protein that binds to DNA and is involved in chromatin formation. It is also secreted from activated macrophages and monocytes and stimulates inflammatory processes by binding to the receptor for advanced glycation end-products (RAGE), TLR-4, and TLR-2 receptors. It has been reported that it can directly penetrate cells and cause oxidative stress. Various studies have shown that HMGB1 plays an important role in the progression of nephropathy, such that its serum and urinary elevation has been used to diagnose DN and its gene expression inhibition has been used to treat nephropathy (45, 46). In this regard, the results of our study showed that in the diabetic group there is a significant increase in HMGB1 expression. On the other hand, our results showed that saffron can suppress its expression. Although no study has reported the effects of total saffron extract on HMGB1 gene expression, other studies have reported that crocin has the ability to suppress HMGB1 gene expression in inflammatory diseases such as (LPS)-induced acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) (47, 48). Overall, these results indicate that HMGB1 plays an important role in inflammatory diseases and could be a target for saffron, although further studies are needed.

Conclusion

In conclusion, this study highlights the significant impact of diabetes on nephropathy, revealing that it is associated with increased expression of inflammatory genes such as TLR-4, S100A8, and HMGB1, along with a disruption of the kidney's antioxidant defense system. Conversely, our findings demonstrate that a 60-day treatment with saffron effectively reduces the expression of these inflammatory genes and alleviates oxidative stress in a dose-dependent manner. These promising results suggest that saffron may serve as a valuable adjunctive therapy for managing diabetes and diabetic nephropathy. Nevertheless, further research is essential to validate these findings and fully understand the therapeutic potential of saffron in this context.

Competing interests

The authors declare that they have no conflicts of interest.

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Author contributions

Jamal Amri & Abbas Alimoradian: Investigation, Methodology and Writing & editing; Hadi Karami, Seied Amirhossein Latifi and Ali Ghazavi: Validation, Methodology; Ahmad Akbari, Zahra Pilevar: Software, Analysis; Abbas Alimoradian: Funding acquisition and project administration.

References

1. Lim A. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361-81. PubMed PMID: 25342915. Pubmed Central PMCID: PMC4206379. Epub 2014/10/25. eng.
2. Selby NM, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. Diabetes Obes Metab. 2020;22(S1):3-15. https://doi.org/10.1111/dom.14007
3. Dwivedi S, Sikarwar MS. Diabetic Nephropathy: Pathogenesis, Mechanisms, and Therapeutic Strategies. Horm Metab Res. 2024 Nov 21. PubMed PMID: 39572154. Epub 2024/11/22. eng.
4. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol. 2017 Dec 7;12(12):2032-45. PubMed PMID: 28522654. Pubmed Central PMCID: PMC5718284. Epub 2017/05/20. eng. https://doi.org/10.2215/CJN.11491116
5. Natesan V, Kim SJ. Diabetic Nephropathy - a Review of Risk Factors, Progression, Mechanism, and Dietary Management. Biomol Ther (Seoul). 2021 Jul 1;29(4):365-72. PubMed PMID: 33888647. Pubmed Central PMCID: PMC8255138. Epub 2021/04/24. eng. https://doi.org/10.4062/biomolther.2020.204
6. Banerjee D, Winocour P, Chowdhury TA, De P, Wahba M, Montero R, et al. Management of hypertension and renin-angiotensin-aldosterone system blockade in adults with diabetic kidney disease: Association of British Clinical Diabetologists and the Renal Association UK guideline update 2021. BMC Nephrol. 2022 Jan 3;23(1):9. PubMed PMID: 34979961. Pubmed Central PMCID: PMC8722287. Epub 2022/01/05. eng. https://doi.org/10.1186/s12882-021-02587-5
7. Sulaiman MK. Molecular mechanisms and therapeutic potential of natural flavonoids in diabetic nephropathy: Modulation of intracellular developmental signaling pathways. Curr Res Pharmacol Drug Discov. 2024 Jan 1;7:100194. https://doi.org/10.1016/j.crphar.2024.100194
8. Ma J, Chadban SJ, Zhao CY, Chen X, Kwan T, Panchapakesan U, et al. TLR4 activation promotes podocyte injury and interstitial fibrosis in diabetic nephropathy. PLoS One. 2014;9(5):e97985. PubMed PMID: 24842252. Pubmed Central PMCID: PMC4026484. Epub 2014/05/21. eng. https://doi.org/10.1371/journal.pone.0097985
9. Tammaro A, Florquin S, Brok M, Claessen N, Butter LM, Teske GJD, et al. S100A8/A9 promotes parenchymal damage and renal fibrosis in obstructive nephropathy. Clin Exp Immunol.

2018 Sep;193(3):361-75. PubMed PMID: 29746703. Pubmed Central PMCID: PMC6150262. Epub 2018/05/11. eng. https://doi.org/10.1111/cei.13154
10. Chen R, Kang R, Tang D. The mechanism of HMGB1 secretion and release. Exp Mol Med. 2022 Feb 1;54(2):91-102. https://doi.org/10.1038/s12276-022-00736-w
11. Sani A, Tajik A, Seiiedi SS, Khadem R, Tootooni H, Taherynejad M, et al. A review of the anti-diabetic potential of saffron. Nutr Metab Insights. 2022;15:11786388221095223. PubMed PMID: 35911474. Pubmed Central PMCID: PMC9335478. Epub 2022/08/02. eng. https://doi.org/10.1177/11786388221095223
12. Bolhassani A. Chapter 10 - Bioactive Components of Saffron and Their Pharmacological Properties. In: Atta ur R, editor. Studies in Natural Products Chemistry. 58: Elsevier; 2018. p. 289-311. https://doi.org/10.1016/B978-0-444-64056-7.00010-6
13. Mahmoudzadeh L, Najafi H, Ashtiyani SC, Yarijani ZM. Anti-inflammatory and protective effects of saffron extract in ischaemia/reperfusion-induced acute kidney injury. Nephrology (Carlton). 2017;22(10):748-54. https://doi.org/10.1111/nep.12849
14. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175-91. https://doi.org/10.3758/BF03193146
15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun;18(6):499-502. PubMed PMID: 4337382. Epub 1972/06/01. eng. https://doi.org/10.1093/clinchem/18.6.499
16. Ashrafi M, AFSAR Z, Erjaee H, Nazifi S. The effects of saffron (Crocus sativus) aqueous extract on TNF-α levels in liver, kidney, and lens tissues of diabetic rats. Turk J Endocrinol Metab. 2018;22(4):217. https://doi.org/10.25179/tjem.2018-59710
17. Karimi-Nazari E, Nadjarzadeh A, Masoumi R, Marzban A, Mohajeri SA, Ramezani-Jolfaie N, et al. Effect of saffron (Crocus sativus L.) on lipid profile, glycemic indices and antioxidant status among overweight/obese prediabetic individuals: A double-blinded, randomized controlled trial. Clin Nutr ESPEN. 2019 Dec;34:130-6. PubMed PMID: 31677703. Epub 2019/11/05. eng. https://doi.org/10.1016/j.clnesp.2019.07.012
18. Karimi E, Oskoueian E, Hendra R, Jaafar HZ. Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules. 2010;15(9):6244-56. https://doi.org/10.3390/molecules15096244
19. Soliman A, Mohamed A, Marie M. Effect of echinochrome on body weight, musculoskeletal system and lipid profile of male diabetic rats. Austin J Endocrinol Diabetes. 2016;3(2):1045.
20. Alaee M, Akbari A, Karami H, Salemi Z, Amri J, Panahi M. Antidiabetic and protective effects of Scrophularia striata ethanolic extract on diabetic nephropathy via suppression of RAGE and S100A8 expression in kidney tissues of streptozotocin-induced diabetic rats. J Basic Clin Physiol Pharmacol. 2020 Jan 22;31(2). PubMed PMID: 31967963. Epub 2020/01/23. eng. https://doi.org/10.1515/jbcpp-2019-0186
21. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Immunomodulatory and antioxidant effects of saffron aqueous extract (Crocus sativus L.) on streptozotocin-induced diabetes in rats. Indian Heart J. 2017 Mar-Apr;69(2):151-9. PubMed PMID: 28460761. Pubmed Central PMCID: PMC5414951. Epub 2017/05/04. eng. https://doi.org/10.1016/j.ihj.2016.09.008
22. Yaribeygi H, Zare V, Butler AE, Barreto GE, Sahebkar A. Antidiabetic potential of saffron and its active constituents. J Cell Physiol. 2019 Jun;234(6):8610-7. PubMed PMID: 30515777. Epub 2018/12/06. eng. https://doi.org/10.1002/jcp.27843
23. Bolhassani A. Bioactive Components of Saffron and Their Pharmacological Properties. Stud Nat Prod Chem. 2018;58:289-311. https://doi.org/10.1016/B978-0-444-64056-7.00010-6
24. Yaribeygi H, Mohammadi MT, Rezaee R, Sahebkar A. Crocin improves renal function by declining Nox-4, IL-18, and p53 expression levels in an experimental model of diabetic nephropathy. J Cell Biochem. 2018 Jul;119(7):6080-93. PubMed PMID: 29575259. Epub 2018/03/27. eng. https://doi.org/10.1002/jcb.26806
25. Altinoz E, Oner Z, Elbe H, Cigremis Y, Turkoz Y. Protective effects of saffron (its active constituent, crocin) on nephropathy in streptozotocin-induced diabetic rats. Hum Exp Toxicol. 2015 Feb;34(2):127-34. PubMed PMID: 24925368. Epub 2014/06/14. eng. https://doi.org/10.1177/0960327114538989
26. Amri J, Alaee M, Latifi SA, Alimoradian A, Salehi M. Amelioration of STZ-induced nephropathy in diabetic rats by saffron hydro alcoholic extract. Horm Mol Biol Clin Investig. 2021 May 21;42(4):411-8. PubMed PMID: 34018383. Epub 2021/05/22. eng. https://doi.org/10.1515/hmbci-2021-0005
27. Ebrahimi F, Aryaeian N, Pahlavani N, Abbasi D, Hosseini AF, Fallah S, et al. The effect of saffron (Crocus sativus L.) supplementation on blood pressure, and renal and liver function in patients with type 2 diabetes mellitus: A double-blinded, randomized clinical trial. Avicenna J Phytomedicine. 2019 Jul-Aug;9(4):322-33. PubMed PMID: 31309071. Pubmed Central PMCID: PMC6612249. Epub 2019/07/17. eng.
28. Milajerdi A, Jazayeri S, Bitarafan V, Hashemzadeh N, Shirzadi E, Derakhshan Z, et al. The effect of saffron (Crocus sativus L.) hydro-alcoholic extract on liver and renal functions in type 2 diabetic patients: A double-blinded randomized and placebo control trial. J Nutr Intermed Metab. 2017 Sep;9:6-11. https://doi.org/10.1016/j.jnim.2017.07.002
29. Abou-Hany HO, Atef H, Said E, Elkashef HA, Salem HA. Crocin mediated amelioration of oxidative burden and inflammatory cascade suppresses diabetic nephropathy progression in diabetic rats. Chem Biol Interact. 2018 Mar 25;284:90-100. PubMed PMID: 29409856. Epub 2018/02/08. eng. https://doi.org/10.1016/j.cbi.2018.02.001
30. Gyurászová M, Kovalčíková AG, Renczés E, Kmeťová K, Celec P, Bábíčková J, et al. Oxidative Stress in Animal Models of Acute and Chronic Renal Failure. Dis Markers. 2019;2019:8690805. PubMed PMID: 30886657. Pubmed Central PMCID: PMC6388331. Epub 2019/03/20. eng. https://doi.org/10.1155/2019/8690805
31. Abbasi-Oshaghi E, Khodadadi I, Mirzaei F, Ahmadi M, Tayebinia H, Goodarzi MT. Anethum graveolens L. Alleviates Sperm Damage by Limiting Oxidative Stress and Insulin Resistance in Diabetic Rats. Open Med Chem J. 2020;14(1). https://doi.org/10.2174/1874104502014010035
32. Samarghandian S, Borji A. Anticarcinogenic effect of saffron (Crocus sativus L.) and its ingredients. Pharmacogn Res. 2014;6(2):99. https://doi.org/10.4103/0974-8490.128963
33. Farahmand SK, Samini F, Samini M, Samarghandian S. Safranal ameliorates antioxidant enzymes and suppresses lipid peroxidation and nitric oxide formation in aged male rat liver. Biogerontology. 2013;14(1):63-71. https://doi.org/10.1007/s10522-012-9409-0
34. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Immuno-

modulatory and antioxidant effects of saffron aqueous extract (Crocus sativus L.) on streptozotocin-induced diabetes in rats. Indian Heart J. 2017;69(2):151-9. https://doi.org/10.1016/j.ihj.2016.09.008

35. Yaribeygi H, Mohammadi MT, Sahebkar A. Crocin potentiates antioxidant defense system and improves oxidative damage in liver tissue in diabetic rats. Biomed Pharmacother. 2018 Feb;98:333-7. PubMed PMID: 29274590. Epub 2017/12/24. eng. https://doi.org/10.1016/j.biopha.2017.12.077
36. Kaur H, Chien A, Jialal I. Hyperglycemia induces Toll like receptor 4 expression and activity in mouse mesangial cells: relevance to diabetic nephropathy. Am J Physiol Renal Physiol. 2012;303(8):F1145-50. https://doi.org/10.1152/ajprenal.00319.2012
37. Zhu T, Meng Q, Ji J, Zhang L, Lou X. TLR4 and Caveolin-1 in monocytes are associated with inflammatory conditions in diabetic neuropathy. Clin Transl Sci. 2017;10(3):178-84. https://doi.org/10.1111/cts.12434
38. Liu S-Y, Nie X-Z, Zhou W-Y, Chen J. Expression and effect of TLR4 in rats with diabetic nephropathy. Asian Pac J Trop Med. 2013;6(8):635-9. https://doi.org/10.1016/S1995-7645(13)60109-1
39. Poursamimi J, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohajeri SA, Mohammadi M. Crocus Sativus (Saffron): An immunoregulatory factor in the autoimmune and non-autoimmune diseases. Iran J Allergy Asthma Immunol. 2020. https://doi.org/10.18502/ijaai.v19i(s1.r1).2852
40. Khedr L, Rahmo RM, Farag DB, Schaalan MF, Hekmat M. Crocin attenuates cisplatin-induced hepatotoxicity via TLR4/NF-κBp50 signaling and BAMBI modulation of TGF-β activity: Involvement of miRNA-9 and miRNA-29. Food Chem Toxicol. 2020;140:111307. https://doi.org/10.1016/j.fct.2020.111307
41. Jin W, Zhang Y, Xue Y, Han X, Zhang X, Ma Z, et al. Crocin attenuates isoprenaline-induced myocardial fibrosis by targeting TLR4/NF-κB signaling: connecting oxidative stress, inflammation, and apoptosis. Naunyn Schmiedebergs Arch Pharmacol. 2020;393:13-23. https://doi.org/10.1007/s00210-019-01704-4
42. Du L, Chen Y, Shi J, Yu X, Zhou J, Wang X, et al. Inhibition of S100A8/A9 ameliorates renal interstitial fibrosis in diabetic nephropathy. Metabolism. 2023;144:155376. https://doi.org/10.1016/j.metabol.2022.155376
43. Chen Z, Chen L, Dai H. Mechanism of resveratrol improving rheumatoid arthritis injury by inhibiting S100A8/A9 expression. Indian J Pharm Sci. 2023;85(2). https://doi.org/10.36468/pharmaceutical-sciences.1077
44. Yang L, Li D, Tang P, Zuo Y. Curcumin increases the sensitivity of K562/DOX cells to doxorubicin by targeting S100 calcium-binding protein A8 and P-glycoprotein. Oncol Lett. 2020;19(1):83-92. https://doi.org/10.3892/ol.2019.11083
45. Liu T, Zhao H, Wang Y, Qu P, Wang Y, Wu X, et al. Serum high mobility group box 1 as a potential biomarker for the progression of kidney disease in patients with type 2 diabetes. Front Immunol. 2024;15:1334109. https://doi.org/10.3389/fimmu.2024.1334109
46. Chen X, Ma J, Kwan T, Stribos EG, Messchendorp AL, Loh YW, et al. Blockade of HMGB1 attenuates diabetic nephropathy in mice. Sci Rep. 2018;8(1):8319. https://doi.org/10.1038/s41598-018-26637-5
47. Ceylan T, Akın A, Kaymak E, Varinli Ş, Toluk A. Crocin Suppresses Inflammatory Response in LPS-Induced Acute Lung Injury (ALI) via Regulation of HMGB1/TLR4 Inflammation Pathway. J Basic Clin Health Sci. 2024;8(2):271-8. https://doi.org/10.30621/jbachs.1233245
48. Zhang D, Qi B-Y, Zhu W-W, Huang X, Wang X-Z. Crocin alleviates lipopolysaccharide-induced acute respiratory distress syndrome by protecting against glycocalyx damage and suppressing inflammatory signaling pathways. Inflamm Res. 2020;69:267-78. https://doi.org/10.1007/s00011-019-01314-z