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The Effect of Crocin and Losartan on Biochemical Parameters and Oxidative Stress Markers in Diabetic Nephropathy Rats Model

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ABSTRACT

Background: Diabetic nephropathy (DN) is the most common cause of the end-stage renal disease (ESRD) globally. This study aimed to evaluate the effect of Crocin and Losartan on DN in diabetic rats.

Methods: A single dose of Streptozotocin (50 mg/kg IP) was administered to 40 male Wistar rats to induce diabetes. Crocin and Losartan (50 and 25 mg/kg, respectively) were given orally for four weeks. The study groups were untreated control, Diabetes, Crocin, Losartan, and Losartan-Crocin. At the end of the intervention, blood was tested for FBS, urea, UA, Cr, albumin, TG, TC, LDL-C, HDL-C, TAC, and MDA.

Results: Serum levels of FBS, urea, UA, TG, TC, and LDL-C increased significantly in the diabetic group compared to the untreated control group (P = 0.001), while albumin and HDL-C decreased significantly (P = 0.001). In the Crocin group, serum FBS, urea, TG, TC, and LDL-C levels were significantly lower than the diabetic group (P = 0.001), while serum albumin levels were significantly higher (P = 0.02). Serum levels of TAC and MDA in the Losartan group increased (P=0.04) and decreased significantly (P = 0.001) compared to the diabetic group, respectively.

Conclusion: The findings of the present study showed that Crocin could control hyperglycemia and prevent DN progression. It appears that combining Losartan with sufficient doses of Crocin improves its efficacy. However, understanding the exact mechanism of these changes requires further studies.

Keywords: Diabetic nephropathy, Crocin, Losartan, Biochemical parameter, Oxidative stress.

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Introduction

Diabetic nephropathy (DN) is the most common cause of kidney failure worldwide. According to the World Diabetes Federation, there will be approximately 463 million people with diabetes worldwide in 2019, which is expected to rise to 578 million by 2030 (1). Diabetic patients develop DN in 20 to 40% of cases (2). Diabetic nephropathy is a serious complication of diabetes, characterized by mild proteinuria that progresses to severe proteinuria and kidney failure (3). Hypertension, thickening of the glomerular basement membrane, and glomerular hyperfiltration lead to enlargement of the mesangial extracellular matrix, resulting in exacerbation of albuminuria and progression of glomerular sclerosis (4). The mechanisms that cause DN to progress are unknown. However, the majority of studies have found a relationship between hyperglycemia and the development of DN. Prolonged hyperglycemia leads to chronic metabolic and hemodynamic changes through the intracellular signal pathways of transcription factors, cytokines, chemokines, growth factors, and the renin-angiotensin system (5). Glycemic control can be effective in reducing the progression of DN and other vascular complications.

On the other hand, renin-angiotensin-aldosterone system (RAAS) blockers and angiotensin receptor blockers (ARBs) have been suggested as standard treatments for hypertension in patients with diabetes and renal failure. The kidney has been shown to contain Ang II type 1 (AT1) receptors, which play essential roles in renal function, long-term blood pressure control, and disease progression (6). Losartan is one of the most clinically experienced AT1 receptor antagonists in the market. Due to the side effects of chemical drugs, the use of herbal medicines as complementary or alternative therapies has been considered by researchers. It is now known that saffron can increase insulin sensitivity and reduce serum glucose levels (7, 8). Crocin is the leading and active biological component of saffron with anti-inflammatory and antioxidant properties (9). This study aimed to evaluate the effect of Crocin separately and in combination with Losartan on DN in an experimental model of Streptozotocin-induced diabetic rats.

Materials and Methods

Streptozotocin (S0130) and Crocin (17304) were purchased from Sigma-Aldrich Company (Sigma brand, USA). Losartan was obtained from ACTOVERCO pharmaceutical factory (Karaj-Iran).

Animal

In this study, 40 male Wistar rats (mean weight: 200-250 gr) were purchased from the Experimental Research Center of Birjand University of Medical Sciences. The study was approved by the Ethics Committee of Birjand University of Medical Sciences (Ethical code: IR.BUMS.REC.1399.019). Before the experiment, the animals had been housed for one week in standard cages to adapt to the environment. Rats had adequate access to water and a standard chow during the study. All cages were kept in a regular light cycle of 12/12 hours of light/darkness and a room temperature between 20 and 26°C.

Experimental design

The animals were randomly divided into five groups (n=8):

1- Untreated control: Non-diabetic rats that received normal saline buffer daily.

2- The diabetic control: Diabetic rats who were given Normal Saline daily.

3- The Crocin-treated group: Diabetic rats who were administered Crocin (50 mg/kg) daily.

4- The Losartan-treated group: Diabetic rats who were administered Losartan (25 mg/kg) daily.

5- The Losartan-Crocin-treated group: Diabetic rats who were administered Losartan + Crocin (25+50 mg/kg, respectively) daily.

Crocin and Losartan were dissolved in Normal Saline and administered via gavage. The treatment started 4 weeks after the induction of diabetes and lasted for 4 weeks.

Diabetes induction

Diabetes was induced by a single intraperitoneal (IP) injection of STZ (50 mg/kg) (10). Streptozotocin (STZ) was dissolved in Sodium Citrate buffer (pH = 4.5, 0.01 M). Rats were given 15% glucose solution for 24 hours to avoid unexpected hypoglycemia. Three days after induction of diabetes, fasting blood sugar (FBS) was measured by a standard glucometer, Accu-Check (Roche, Germany), from the tail vein. Rats with FBS >250 mg/dL were considered diabetic rats.

Sample collection

The 24-hour urine was collected in metabolic cages at the end of the experiment to analyze urinary total protein (UTP). Then, rats were anesthetized intraperitoneally with Sodium Thiopental (50 mg/kg). Blood samples were taken from each rat's heart. The tube containing blood was incubated for 40 min at room temperature, and then, the serum was separated by centrifuging at 3000 rpm for 10 min.

Measurement of biochemical parameters

Biochemical parameters of albumin, urea, creatinine (Cr), uric acid (UA), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), and UTP were measured by standard kits (Pars Azmun Corp., Iran) and an automatic analyzer (Prestige 24i, Japan).

Measurement of total antioxidant capacity (TAC)

Total antioxidant capacity was measured using commercial kits (Zantox-Iran). Benzie and strain methods were used to measure rat's serum TAC by FRAP (11). For this purpose, at first, the working FRAP reagent was prepared as follows. 25 ml of acetate buffer (pH = 6.3, 300 mmol/l) was mixed with 2.5 ml of TPTZ solution and 2.5 ml of iron chloride. 5 µL of a serum sample, 5 µL of standard solution (Trolox, concentrations of 62.5, 125, 250, 500, and 1000 µmol) were added in a microplate. 5 µL of distilled water was added as blanks. Then, 200 µL of FRAP reagent were added to all wells. Incubation was done for 15 min at 37°C, and then, read at a wavelength of 593 nm by an absorption spectrophotometer.

Measurement of serum Malondialdehyde (MDA)

Malondialdehyde was measured using commercial kits (Zantox, Iran). Thiobarbituric Acid Reactive substances (TBARs) method was used to measure the serum level of MDA. In this method, TBA binds to MDA and produces a color complex MDA(TBA)2, which is purple. 100 µl of MDA solution as standard (concentrations of 0.156, 1.312, 0.625, 1.25, 2.5, 5, and 10), serum and control samples were added into separate tubes. Then, 1000 µl of TBARs reagent and 10 µl of BHT solution were added. The mixture was heated at 95°C for 20 min, and then, placed in an ice bath for 10 min. Then, 1100 µl of n-butanol was added. The tubes were centrifuged at 2000 rpm for 20 min. 250 µl of supernatant were removed for MDA assay, and its absorbance was recorded at 532 nm.

Statistical analysis

The statistical analysis was performed by SPSS version 16. For comparison between groups, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used. The paired sample t-test was used for each group before and after compassion. Data are expressed as Mean±SEM. Statistical significant level was considered at $P<0.05$.

Results

Effect of Crocin and Losartan on FBS

The levels of FBS before and after the intervention are shown in Table 1. When compared to the untreated control group, FBS levels increased significantly in all diabetic groups before the intervention (P=0.001). Post-intervention comparison between groups showed that FBS levels were lower in the treated groups than in the diabetic group. These differences were significant in the Crocin-treated group (P=0.001).

Intragroup comparison before and after the intervention showed that FBS levels decreased in the treated groups while it increased in the diabetic group, but the increase was not statistically significant.

Effect of Crocin and Losartan on body weight and kidney/body weight ratio

Table 1 shows the mean body weight and kidney/body weight ratio. Before the intervention, the results showed a significant decrease in body weight in all diabetic rats compared to the untreated control group (P=0.001). After the intervention, intergroup comparisons showed that body weight increased in the treated groups compared to the diabetic group, but the differences were not significant.

Also, the kidney weight/body weight ratio showed a significant increase in the diabetic group compared to the untreated control group (P=0.001). The mean of this index decreased in the groups treated with Crocin and Losartan-Crocin compared to the diabetic group, but the differences were not significant.

Table 1. The effect of Crocin and Losartan on FBS, body weight, and kidney/body weight ratio

Parameters	Untreated	Diabetic	Crocin	Losartan	Losartan-Crocin
FBS before intervention (mg/dl)	89.88±2.09	407±18.30*	387.38±6.34*	398.71±30.06*	422.57±22.49*
FBS after intervention (mg/dl)	91.50±1	433.14±20.14*	336.62±13.53*#	386.43±15.53*	374.14±23.21*
Body weight (g) before intervention	317.25±8.75	205.71±6.40*	196.50±7.68*	196.43±6.04*	221.43±8.29*
Body weight (g) after intervention	339.62±9.06	187.71±4.65*	192.38±8.67*	179.71±9.52*	208.29±11.09*
kidney/body weight ratio (g)	0.69±0.02	1.31±0.09*	1.28±0.05*	1.30±0.03*	1.23±0.04*

Data are presented as Mean ± SEM. Changes of FBS (mg/dl), body weight, and kidney/body weight ratio in groups of untreated (non-diabetic), Diabetic, Crocin (Diabetic treated with Crocin), Losartan (Diabetic treated with Losartan), Losartan-Crocin (Diabetic treated with Losartan-Crocin), n = 8. Data are presented as Mean ± SEM.

* P<0.05: Significant differences versus untreated control group.

# P<0.05: Significant differences versus diabetic group.

Effect of Crocin and Losartan on the 24-hour urine volume and urinary total protein (UTP)

Figure 1 shows the 24-hour urine volume and UTP before and after the intervention. Before the intervention, the results showed a significant increase in the 24-hour urine volume and UTP in all diabetic rats compared to the untreated control group (P=0.001).

After the intervention, intra-group comparisons showed that the 24-hour urine volume and UTP decreased in the treated groups compared to the diabetic group, although the differences were not statistically significant.

Intragroup comparison before and after the intervention showed that UTP decreased significantly (P=0.001) in the treated groups while it increased significantly (P=0.02) in the diabetic group.

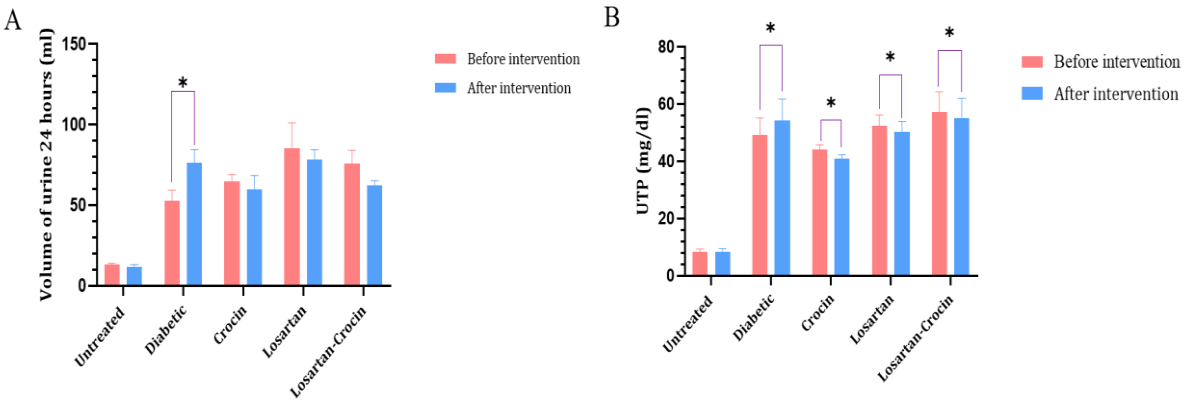

Figure 1. Changes of A: 24-hour urine volume (ml), and B: Urinary total protein (UTP) (mg/dl) in groups of untreated (non-diabetic), Diabetic, Crocin (Diabetic treated with Crocin), Losartan (Diabetic treated with Losartan), Losartan-Crocin (Diabetic treated with Losartan-Crocin), n = 8. P<0.05: Significant differences versus untreated control group.

Effect of Crocin and Losartan on serum levels of urea, creatinine (Cr), and uric acid (UA)

Figure 2 shows serum levels of urea, Cr, and UA. In this study, the mean serum level of urea increased significantly in the diabetic group compared to the untreated control group (P=0.001). Serum urea levels were significantly reduced in the Crocin-treated group compared to the diabetic group (P=0.001). Also, the serum urea level decreased in the Losartan-Crocin-treated group compared to the Losartan-treated group, but the decrease was not statistically significant.

Serum Cr levels also increased in the diabetic groups compared to the untreated control group. Cr decreased in all treated groups (Crocin, Losartan, and Losartan- Crocin) compared to the diabetic group, but these changes were insignificant (P=0.74, P=0.99, P=0.92, respectively).

Serum UA levels increased significantly in the diabetic group compared to the untreated control group (P=0.006). The serum levels of UA in the Crocin, Losartan, and Losartan-Crocin groups decreased compared to the diabetic group, but the differences were not significant (P=0.07, P=0.99, P=0.73, respectively). Also, the serum UA level dropped in the Losartan-Crocin-treated group compared to the Losartan-treated group, but it was not statistically significant.

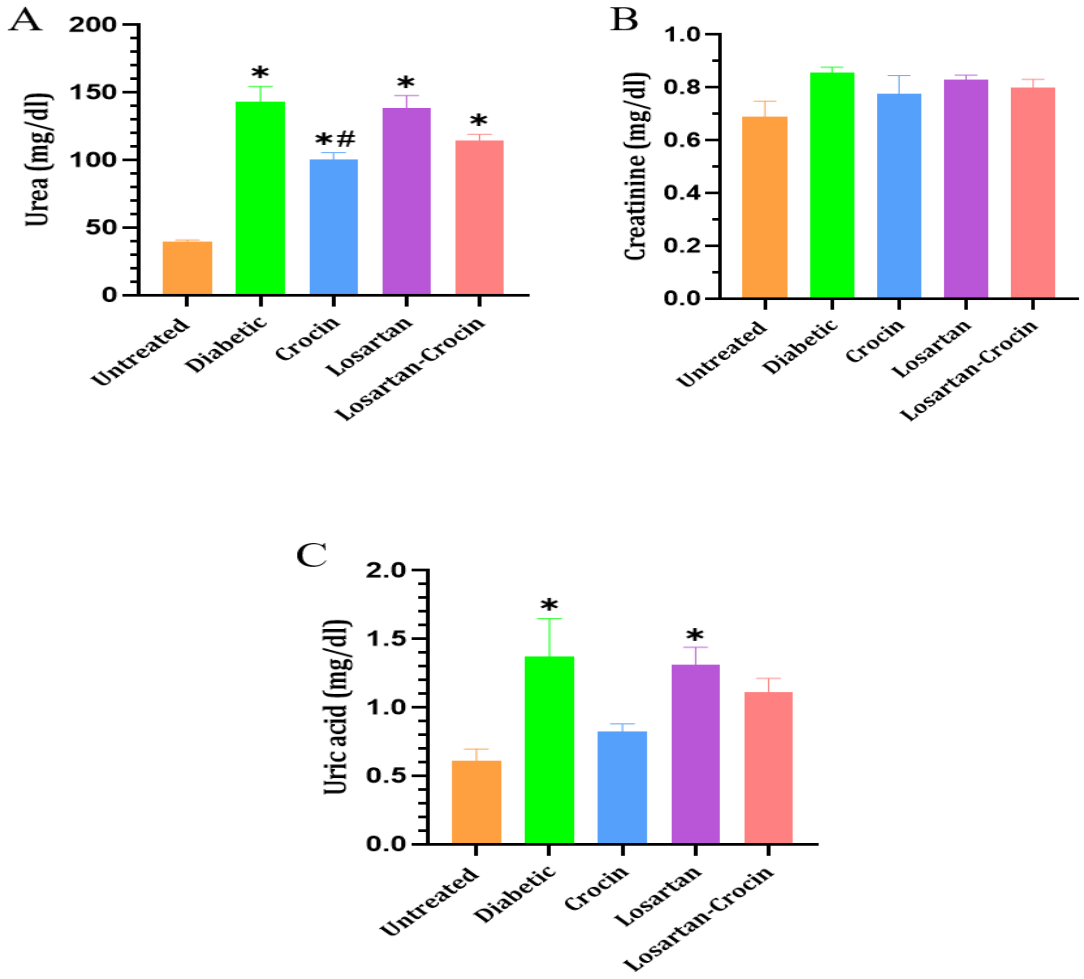

Figure 2. Changes of A: Urea, B: Creatinine and, C: Uric acid (mg/dl) in the groups of untreated (non-diabetic), Diabetic, Crocin (Diabetic treated with Crocin), Losartan (Diabetic treated with Losartan), Losartan-Crocin (Diabetic treated with Losartan-Crocin), n = 8. Data are presented as Mean ± SEM.
*P<0.05: Significant differences versus Untreated control group.

Effect of Crocin and Losartan on serum level of albumin

Table 2 shows the mean serum level of albumin. The present study results showed that the serum albumin levels were significantly reduced in the diabetic group compared to the untreated control group (P=0.001). In the groups treated with Crocin and Losartan-Crocin, the serum level of albumin increased significantly compared to the diabetic group (P=0.001, P=0.01, respectively). Furthermore, serum albumin levels increased in the Losartan-Crocin group compared with the Losartan group but were not statistically significant.

Effect of Crocin and Losartan on serum levels of lipid profile

Table 2 shows the mean serum levels of lipid profile. The results of the present study showed that the serum levels of TG, LDL-C, and TC increased significantly in the diabetic group compared to the untreated control group (P=0.001). In contrast, serum levels of TG, LDL-C, and TC were significantly reduced in the Crocin-treated group compared to the diabetic group (P=0.001, P=0.001, P=0.03, respectively).

Serum HDL-C levels in the diabetic group decreased significantly compared to the untreated control group (P=0.001) and increased significantly in the Crocin-treated group compared to the diabetic group (P=0.02).

Effect of Crocin and Losartan on serum levels of oxidative stress markers

Table 2 shows the mean serum levels of oxidative stress markers. Serum TAC levels decreased in the diabetic group compared to the untreated control group, but this change was insignificant (P=0.21). In the Losartan group, serum TAC level increased significantly compared to the diabetic group (P=0.001). Also, serum TAC levels in groups treated with Crocin and Losartan-Crocin increased compared to the diabetic group, but these changes were not statistically significant (P=0.56, P=0.37, respectively).

The level of MDA, an indicator of lipid peroxidation, increased in the diabetic group compared to the untreated control group, but this change was not significant (P=1.00). Serum levels of MDA were significantly decreased in the Losartan group compared to the diabetic group (P=0.04). Furthermore, MDA levels were reduced in the Crocin-treated and Losartan-Crocin-treated groups compared to the diabetic group, but the differences were not statistically significant (P=0.71, P=0.86, respectively).

Table 2. The effect of Crocin and Losartan on serum levels of albumin, lipid profile (TG, LDL-C, HDL-C, and TC), and oxidative stress markers (TAC and MDA)

Parameters	Untreated	Diabetic	Crocin	Losartan	Losartan-Crocin
Albumin (mg/dl)	3.17±0.08	2.27±0.09*	2.88±0.06#	2.52±0.011*	2.71±0.07*#
TG (mg/dl)	39.27±1.43	73.71±2.08*	57.12±2.35*#	67.42±3*	63.85±3.01*
LDL-C (mg/dl)	9±0.42	18.42±0.52*	12±0.7#	16.28±1.4*	14.57±1.61*
HDL-C (mg/dl)	30.25±0.75	18.85±1.48*	24.25±1.29*#	20.14±1.18*	22.42±1.15*
TC (mg/dl)	38.43±1.6	52±2.83*	43.37±1.46#	45.71±2.51	43.5±1.67
MDA (µMol/L)	1.1±0.05	1.21±0.08	1.08±0.07	0.91±0.08#	1.11±0.05
TAC (µMol/L)	391.38±9.13	338.14±9.61	375±12.86	455.71±27.17#	384.14±22.79

Changes of albumin, lipid profile, and oxidative stress markers in the groups of untreated (non-diabetic), Diabetic, Crocin (Diabetic treated with Crocin), Losartan (Diabetic treated with Losartan), Losartan-Crocin (Diabetic treated with Losartan-Crocin), n = 8. Data are presented as Mean ± SEM.

* P<0.05: Significant differences versus Untreated control group.

# P<0.05: Significant differences versus Diabetic group.

Discussion

DN is a common problem in diabetic patients. The use of medicinal plants in the treatment of this disease and its complications has been discussed from the past to the present, but no definite evidence has been found about the effectiveness of many of them. This study aimed to evaluate the effect of Crocin separately and in combination with Losartan on biochemical factors and oxidative stress markers in diabetic rats. The results of this study generally showed that Crocin separately and in combination with Losartan improved biochemical parameters, body weight, and kidney/body weight ratio compared to the diabetic group. On the other hand, using Crocin with Losartan to some extent increased the effectiveness of Losartan in improving hyperglycemic conditions.

The present study showed that daily administration of Crocin (50 mg/kg) significantly reduced FBS levels compared to the diabetic group, which is consistent with the results of previous studies (12, 13). Crocin has been shown to have hypoglycemic effects by increasing peripheral insulin sensitivity. Crocin increases insulin sensitivity by activating two signaling pathways of insulin-dependent (phosphatidylinositol-3 kinase/protein kinase B), insulin-independent (5-AMP-activated protein kinase/acetyl-COA carboxylase

(AMPK/ACC) and mitogen-activated protein kinases (MAPKs) pathways (14, 15). The current and earlier findings of the study suggest that Crocin has hypoglycemic properties.

After diabetes induction, the bodyweight of diabetic rats begins to decline. Weight loss is a side effect of diabetes because the body cannot utilize the extra glucose generated during gluconeogenesis (16). This study showed that the diabetic rats treated with Crocin and Losartan-Crocin had the lowest rates of weight loss after the intervention, while diabetic rats had the highest rates of weight loss. This may be due to the ability of Crocin to improve insulin sensitivity and hypoglycemic effects (17). Crocin increased the effectiveness of Losartan in improving weight loss.

DN is characterized by proteinuria. The results of the present study showed that UTP was significantly increased in the diabetic group compared to the untreated control group. The most important risk factors for proteinuria are hyperglycemia and high blood pressure (18). UTP was remarkably reduced in the diabetic groups treated with Crocin and Losartan. Crocin lowered urine protein excretion by reducing hyperglycemia and oxidative stress, according to Hadeer O *et al*. (19). Losartan also decreases blood pressure, which helps reduce proteinuria, as demonstrated by Lee *et al.* (20). As a result, the present study demonstrates the beneficial effects of Crocin and Losartan's in preventing DN development.

The RAAS system is activated in hyperglycemic conditions, which increases NO production and causes endothelial damage. As a result, the kidneys lose their ability to filter blood for creatinine and other nitrogen components, resulting in higher serum levels of urea, Cr, and UA (19, 21). The present study showed that in the diabetic group compared with the untreated control group, serum levels of urea and Cr increased significantly, which is consistent with the results of the study of Amin *et al*. (22). The present study showed that Crocin could reduce serum urea levels (significantly), Cr, and UA (non-significant) during chronic hyperglycemia, which is consistent with the results of the study of Altinoz *et al.* (23). Crocin enhances the antioxidative defense system, inhibits inflammation, and reduces apoptosis, all of which may help improve kidney function indicators (24). Furthermore, Crocin improved Losartan efficacy on renal function parameters.

Albumin is the most abundant soluble protein in the blood, and the liver produces it almost exclusively. Insulin is an important regulator of albumin (25). The results of this study demonstrated that serum albumin levels in the diabetic group's were significantly lower than those in the non-diabetic control group, which is consistent with the findings of Zhang *et al*. (26). Hyperglycemia and oxidative stress exacerbate β-cell dysfunction and deplete insulin secretory reserve, causing serum albumin levels to decrease (26). Iwasaki *et al.* (27) reported that serum albumin levels in patients with DN are associated with proteinuria. The present study showed that Crocin could significantly increase serum albumin levels. El-Fawal *et al.* observed that Crocin reduces oxidative stress and inflammatory factors induced by hyperglycemia, resulting in decreased albumin excretion by the kidneys (21). Also, it was reported that the treatment with RAAS inhibitors was beneficial not only in reducing albuminuria but also in maintaining serum albumin levels (26). The present study showed that Losartan-Crocin treatment significantly increased serum albumin levels compared to the diabetic group. This indicates that Crocin has enhanced Losartan's efficacy on serum albumin levels.

Reports indicate that dyslipidemia is one of the most common and most anticipated features of diabetes due to decreased insulin or insulin resistance (28). The results of the present study showed that compared to the diabetic group, Crocin significantly decreased serum levels of TG, LDL-C, and TC while it increased serum level of HDL-C significantly. In a study conducted by Sefidgar *et al.* (15), a dose of 60 mg/kg Crocin was found to be more efficient than a dose of 40 mg/kg in changing lipid profiles in diabetic rats. Crocin has been shown to be involved in the mechanism of crocin fat reduction by inhibiting pancreatic lipase (29). Crocin has enhanced Losartan's efficacy on lipid profile.

Prolonged hyperglycemia is associated with an increase in oxygen-free radicals (30). According to the findings of the present study, the serum level of MDA increased, and TAC decreased in the diabetic group compared to the untreated control group (non-significant). Compared to the diabetic group, serum TAC levels in the Losartan-treated group increased significantly, while MDA levels decreased significantly. By blocking the angiotensin-II receptor, Losartan may decrease oxidative stress

and ameliorate diabetes-related oxidative damage (31, 32), which is consistent with the findings of Ateyya *et al*. They reported that Losartan strengthens the antioxidant defense system in diabetic rats (33).

Strengths and limitations

The present study had several strengths. First, to the best of our knowledge, this is the first study that investigated the effect of Crocin and Losartan on DN. Second, because it predicted mortality, 8 rats were placed in each group. Third, the intervention was started 4 weeks after induction of diabetes. Fourth, the effect of Losartan and Crocin was investigated.

The present study also has several limitations. First, a single dose of the drug was used due to the large number of rats and groups studied and the high costs. Secondly, the treatment period was shortened due to the mortality of diabetic rats.

Conclusion

The findings of the present study showed that Crocin separately was able to significantly improve the serum levels of glucose, urea, albumin, and lipid profile. At the same time, Crocin, in combination with Losartan, was able to improve serum levels of glucose, albumin, renal function tests (urea, Cr, and UA), and lipid profile compared to the Losartan-treated group (non-significant). Consequently, it is suggested that combining Crocin with chemical medications in the treatment and prevention of DN may lead to better results and less drug side effects. However, further studies are required to understand the exact mechanism of these changes.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contributions

ARF conceived the initial idea for the study and helped to study design. YM performed experiments and data collection. FS performed the statistical analysis. MZ acted as the scientific advisor. All authors read and approved the final manuscript.

Ethical issues

The study was approved by the Ethics Committee of Birjand University of Medical Sciences (Ethical code: IR.BUMS.REC.1399.019).

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References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019; 157:107843. doi: 10.1016/j.diabres.2019.107843.
2. Baek JH, Lee WJ, Lee BW, Kim SK, Kim G, Jin SM, et al. Age at diagnosis and the risk of diabetic nephropathy in young patients with type 1 diabetes mellitus. Diabetes Metab J. 2021; 45(1):46-54. doi: 10.4093/dmj.2019.0134.
3. Sagoo MK, Gnudi L. Diabetic nephropathy: An overview. Methods Mol Biol. 2020; 2067:3-7. doi: 10.1007/978-1-4939-9841-8_1.
4. Papadopoulou-Marketou N, Paschou SA, Marketos N, Adamidi S, Adamidis S, Kanaka-Gantenbein C. Diabetic nephropathy in type 1 diabetes. Minerva Med. 2018; 109(3):218-28. doi: 10.23736/S0026-4806.17.05496-9.
5. Zhang L, Wen Z, Han L, Zheng Y, Wei Y, Wang X, et al. Research progress on the pathological mechanisms of podocytes in diabetic nephropathy. J Diabetes Res. 2020; 7504798. doi: 10.1155/2020/7504798.
6. Rianto F, Hoang T, Revoori R, Sparks MA. Angiotensin receptors in the kidney and vasculature in hypertension and kidney disease.

Mol Cell Endocrinol. 2021; 529:111259. doi: 10.1016/j.mce.2021.111259.

7. Koszegi S, Molnar A, Lenart L, Hodrea J, Balogh DB, Lakat T, et al. RAAS inhibitors directly reduce diabetes-induced renal fibrosis via growth factor inhibition. J Physiol. 2019; 597(1):193-209. doi: 10.1113/JP277002.

8. Pechlivanova D, Krumova E, Kostadinova N, Mitreva-Staleva J, Grozdanov P, Stoynev A. Protective effects of losartan on some type 2 diabetes mellitus-induced complications in Wistar and spontaneously hypertensive rats. Metab Brain Dis. 2020; 35(3):527-38. doi: 10.1007/s11011-020-00534-1.

9. Hussain MA, Abogresha NM, AbdelKader G, Hassan R, Abdelaziz EZ, Greish SM. Antioxidant and anti-inflammatory effects of crocin ameliorate doxorubicin-induced nephrotoxicity in rats. Oxid Med Cell Longev. 2021; 2021:8841726. doi: 10.1155/2021/8841726.

10. Elsherbiny NM, El-Sherbiny M, Said E. Amelioration of experimentally induced diabetic nephropathy and renal damage by nilotinib. J Physiol Biochem. 2015; 71(4):635-48. doi: 10.1007/s13105-015-0428-6.

11. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry. 1996; 239(1):70-6. doi: 10.1006/abio.1996.0292.

12. Asri-Rezaei S, Tamaddonfard E, Ghasemsoltani-Momtaz B, Erfanparast A, Gholamalipour S. Effects of crocin and zinc chloride on blood levels of zinc and metabolic and oxidative parameters in streptozotocin-induced diabetic rats. Avicenna J Phytomed. 2015; 5(5):403-12. PMID: 26468459.

13. Samarghandian S, Azimi-Nezhad M, Borji A, Farkhondeh T. Effect of crocin on aged rat kidney through inhibition of oxidative stress and proinflammatory state. Phytother Res. 2016; 30(8):1345-53. doi: 10.1002/ptr.5638.

14. Kang C, Lee H, Jung ES, Seyedian R, Jo M, Kim J, et al. Saffron (Crocus sativus L.) increases glucose uptake and insulin sensitivity in muscle cells via multipathway mechanisms. Food Chem. 2012; 135(4):2350-8. doi: 10.1016/j.foodchem.2012.06.092.

15. Sefidgar SM, Ahmadi-Hamedani M, Jebelli Javan A, Narenji Sani R, Javaheri Vayghan A. Effect of crocin on biochemical parameters, oxidative/antioxidative profiles, sperm characteristics and testicular histopathology in streptozotocin-induced diabetic rats. Avicenna J Phytomed. 2019; 9(4):347-61. PMID: 31309073.

16. Talebanzadeh S, Ashrafi M, Kazemipour N, Erjaee H, Nazifi S. Evaluation of the effects of saffron aqueous extract on oxidative stress in the lens of streptozotocin-induced diabetic rats. Biomedical Research and Therapy. 2018; 5(4):2133-41. doi:10.15419/bmrat.v5i4.427.

17. 17.Yaribeygi H, Noroozadeh A, Taghi-Mohammadi M, Johnston TP, Sahebkar AH. Crocin improves oxidative stress by potentiating intrinsic Anti-Oxidant defense systems in pancreatic cells during uncontrolled hyperglycemia. Pharmacopuncture. 2019; 22(2):83-9. doi: 10.3831/KPI.2019.22.010.

18. Michishita R, Matsuda T, Kawakami S, Tanaka S, Kiyonaga A, Tanaka H, et al. Hypertension and hyperglycemia and the combination thereof enhances the incidence of chronic kidney disease (CKD) in middle-aged and older males. Clin Exp Hypertens. 2017; 39(7):645-54. doi: 10.1080/10641963.2017.1306541.

19. 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; 284:90-100. doi: 10.1016/j.cbi.2018.02.001.

20. Lee YJ, Cho S, Kim SR, Jang HR, Lee JE, Huh W, et al. Effect of losartan on proteinuria and urinary angiotensinogen excretion in non-diabetic patients with chronic kidney disease. Postgrad Med J. 2011; 87(1032):664-9. doi: 10.1136/pgmj.2011.118059.

21. El-Fawal R, El Fayoumi HM, Mahmoud MF. Diosmin and crocin alleviate nephropathy in metabolic syndrome rat model: Effect on oxidative stress and low grade inflammation. Biomed Pharmacother 2018; 102:930-7. doi: 10.1016/j.biopha.2018.03.162.

22. Amin SN, El-Gamal EM, Rashed LA, Kamar SS, Haroun MA. Inhibition of notch signalling and mesangial expansion by combined glucagon like peptide-1 agonist and crocin therapy in animal model of diabetic nephropathy. Arch Physiol Biochem. 2020: 1-11. doi: 10.1080/13813455.2020.1846203.

23. 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; 34(2):127-34. doi: 10.1177/0960327114538989.

24. Yaribeygi H, Mohammadi MT, Rezaee R, Sahebkar AH. 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; 119(7):6080-93. doi: 10.1002/jcb.26806.

25. Chang DC, Xu X, Ferrante-Jr AW, Krakoff J. Reduced plasma albumin predicts type 2 diabetes and is associated with greater adipose tissue macrophage content and activation. Diabetol Metab Syndr. 2019; 11(1):14. doi: 10.1186/s13098-019-0409-y.

26. Zhang J, Zhang R, Wang Y, Li H, Han Q, Wu Y, et al. The level of serum albumin is associated with renal prognosis in patients with diabetic nephropathy. J Diabetes Res. 2019; 2019:7825804. doi: 10.1155/2019/7825804.

27. Iwasaki T, Togashi Y, Terauchi Y. Significant association of serum albumin with severity of retinopathy and neuropathy, in addition to that of nephropathy, in Japanese type 2 diabetic patients. Endocr J. 2008; 55(2):311-6. doi: 10.1507/endocrj.k07-086.

28. Nandini HS, Naik PR. Action of corilagin on hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. Chem Biol Interact. 2019; 299:186-93. doi: 10.1016/j.cbi.2018.12.012.

29. Naserizadeh SK, Taherifard MH, Shekari M, Mesrkanlou HA, Asbaghi O, Nazarian B, et al. The effect of crocin supplementation on lipid concentrations and fasting blood glucose: A systematic review and meta-analysis and meta-regression of randomized controlled trials. Complement Ther Med. 2020; 52:102500. doi: 10.1016/j.ctim.2020.102500.

30. Fajriana H, Farmawati A, Arsanti-Lestari L. Antioxidant effect of purple eggplant flour (solanum melongena l.) against oxidative stress in hyperglycaemic rats. Romanian Journal of Diabetes Nutrition and Metabolic Diseases. 2017; 24(3):247-54. doi:10.1515/rjdnmd-2017-0030.

31. Nair AR, Ebenezer PJ, Saini Y, Francis J. Angiotensin II-induced hypertensive renal inflammation is mediated through HMGB1-TLR4 signaling in rat tubulo-epithelial cells. Exp Cell Res. 2015; 335(2):238-47. doi: 10.1016/j.yexcr.2015.05.011.

32. Shirai A, Yamazaki O, Horita S, Nakamura M, Satoh N, Yamada H, et al. Angiotensin II dose-dependently stimulates human renal proximal tubule transport by the nitric oxide/guanosine 3',5'-cyclic monophosphate pathway. J Am Soc Nephrol. 2014; 25(7):1523-32. doi: 10.1681/ASN.2013060596.

33. Ateyya H, Nader MA, El-Sherbeeny NA. Beneficial effects of rosiglitazone and losartan combination in diabetic rats. Can J Physiol Pharmacol. 2018; 96(3):215-20. doi: 10.1139/cjpp-2017-0332.