www.nephropathol.com Doi: 10.15171/jnp.2019.08 J Nephropathol. 2019;8(1):e08

Journal of Nephropathology

Does curcumin or metformin attenuate oxidative stress and diabetic nephropathy in rats?

Soheila Asadi[1], Mohammad Taghi Goodarzi[1], Jamshid Karimi[1], Mohammad Hashemnia[2], Iraj Khodadadi[1*]

[1]Department of Clinical Biochemistry, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 29 July 2018
Accepted: 14 November 2018
Published online: 3 December 2018

Keywords:
Curcumin
Diabetic nephropathy
Metformin
Oxidative stress
Diabetic kidney disease
Reactive oxygen species

ABSTRACT

Background: Since the importance of oxidative stress in the development of diabetic nephropathy (DN) has previously been established, the therapeutic effects of various natural antioxidant agents or synthetic drugs have so far been investigated.

Objectives: The aim of this study was to investigate the beneficial effects of curcumin (a natural polyphenol) and metformin (a common therapeutic medicine for type 2 diabetes) on oxidative status in kidney of type 1 diabetic rats.

Materials and Methods: In this experimental study 60 male Wistar rats were divided into 10 groups. Type 1 diabetes was induced by streptozotocin. Rats received chow diet and treated with either normal saline in control (N) and diabetic control (D) groups or different doses of metformin (Met) (300 or 500 mg/kg body weight) or curcumin (Cur) (50 or 150 mg/kg body weight) in N+Met300, N+Met500, N+Cur50, N+Cur150, D+Met300, D+Met500, D+Cur50, and D+Cur150 groups. Urinary creatinine, urea, and protein were measured. Total antioxidant capacity (TAC), total oxidant status (TOS), malondialdehyde (MDA), and the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were assessed in kidney tissues.

Results: Both metformin and curcumin showed significant effects on urinary creatinine, urea, and protein levels (P value for all was <0.001). Unlike metformin, curcumin completely restored TAC and TOS ($P<0.001$), and MDA ($P=0.012$) in kidney tissues and significantly recovered the activities of SOD ($P=0.003$), GPx ($P<0.001$), and catalase ($P=0.011$).

Conclusions: Curcumin was found more effective than metformin in attenuating oxidative status in DN.

Implication for health policy/practice/research/medical education:
It has been shown that antioxidant agents might be useful in attenuating oxidative stress induced by DN. The present study comparatively investigated antioxidant properties of metformin and curcumin in kidney tissue of diabetic rats. Our results showed that although both metformin and curcumin could improve traditional biomarkers associated with DN, curcumin was more effective than metformin in reduction of oxidative stress in treated groups. Therefore, curcumin exhibited advantages on metformin and can be considered as a candidate therapeutic agent with potential anti-oxidative properties.

Please cite this paper as: Asadi S, Goodarzi MT, Karimi J, Hashemnia M, Khodadadi I. Does curcumin or metformin attenuate oxidative stress and diabetic nephropathy in rats?. J Nephropathol. 2019;8(1):e08. Doi: 10.15171/jnp.2019.08.

Original Article

1. Background

Diabetes is one of the most important metabolic disorders which contribute to a high morbidity and mortality worldwide (1). Recent studies on diabetes have demonstrated that hyperglycemia increases production of reactive oxygen species (ROS) in the electron transport chain and induces oxidative stress (1,2) which in turn plays a crucial role in the development of diabetes-related complications such as diabetic nephropathy (DN) (2). DN (diabetic kidney disease) is one of the most important microvascular complications associated with diabetes that affects kidneys and leads to the end-stage renal disease (3,4). In oxidative stress, redox equilibrium is altered due to the elevation of ROS production or inadequate antioxidant defence. These conditions result in the structural damages in DNA, RNA, lipids, and proteins and yields to glomerular and tubular hypertrophy. The thickening of glomerular and tubular membranes ultimately leads to the cellular apoptosis or necrotic cell death and eventually leads to glomerular dysfunction characterized by albuminuria, proteinuria, glomerulosclerosis, and tubule-interstitial fibrosis (5).

Due to the importance of oxidative stress in the

*Corresponding author: Iraj Khodadadi; Email: khodadadi@umsha.ac.ir

development of complications associated with diabetes, the therapeutic effects of various natural antioxidant agents including vitamin A, C and E (6) or synthetic drugs such as metformin have been investigated so far (7,8). Metformin is a biguanide derivative with hypoglycemic effect which is widely used for the treatment of type 2 diabetes mellitus (T2DM) (9).

Since the antioxidant property of metformin has also been shown through the impact on SIRT1, NF-kb and mild inhibition of respiratory complex I (10-12), it is hypothesized that metformin may beneficially improve oxidative status in kidneys and protects the development of DN (13). However, a restricted use of metformin is recommended because of its association with increased mortality (8).

Curcumin, a lipophilic polyphenol with powerful hypoglycemic effect, is a major component of turmeric, which has been widely used in many countries for therapeutic purposes (14). Due to the presence of several chemically active keto/enol groups in curcumin structure, this compound shows antioxidant properties (14). Curcumin could affect the activity of SIRT1 and decrease transcription of NF-kb (15,16), thereby attenuates oxidative stress. Moreover, curcumin is a powerful activator of nuclear factor erythroid 2-related factor 2 (Nrf2), that binds to antioxidant response elements (AREs) promoter and increases transcription of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (15). Taken together, curcumin could be useful in the treatment of diabetic kidney disease due to its powerful antioxidant properties.

2. Objectives

The aim of the present study was to compare the effects of metformin and curcumin on urine volume and urinary creatinine, urea, and protein levels, and oxidative status in kidney tissue of diabetic rats.

3. Material and Methods

This experimental study was conducted from October 2016 to June 2017 in the research laboratory of clinical biochemistry department (Hamadan University of Medical Sciences, Hamadan, Iran). Sixty male Wistar rats (8 weeks old, weighing 240-260 g) were used in the present study. Animals were purchased from the university animal house (Hamadan, Iran) and were housed in standard plastic cages (4 per cage), with 12-hour light and dark cycle, temperature 22±2˚C and free access to water and standard chow diet. The simple randomization method was used for assignment of the rats in different groups (17) as following and received standard chow diet. Metformin was orally (gavages) given to the rats at two different concentrations, 300 and 500 milligram per kilogram of body weight (mg/kg bw) (18), while for curcumin the 50 and 150 mg/kg bw concentrations were used (19).

Groups were, (i). Normal (N): healthy control group; (ii). N+MET300: normal healthy rats treated with 300 mg/kg.bw of metformin; (iii). N+MET500: control rats treated with 500 mg/kg bw of metformin; (iv). N+Cur50: control rats treated with 50 mg/kg.bw of curcumin; (v). N+Cur150: control rats treated with 150 mg/kg bw of curcumin; (vi). Diabetic (D): diabetic untreated group; (vii). D+MET300: diabetic group treated with 300 mg/kg bw of metformin; (iix). D+MET500: diabetic group treated with 500 mg/kg.bw of metformin, (ix). D+Cur50: diabetic group treated with 50 mg/kg bw of curcumin; (x). D+Cur150: diabetic group treated with 150 mg/kg bw of curcumin.

3.1. Induction of diabetes

To induce diabetes, fasted overnight rats in diabetic groups (Diabetic, D+MET300, D+MET500, D+Cur50 and D+Cur150) were intraperitoneally injected streptozotocin (STZ) (Santa Cruz Biotechnology, Heidelberg-Germany), dissolved in 0.1 M sodium citrate buffer pH:4.5. The remaining rats in the other groups (Normal, N+MET300, N+MET500, N+Cur50 and N+Cur150) received the same volumes of 0.1 M sodium citrate buffer as carrier. Seventy-two hours after injection of STZ, fasting blood glucose level was assessed using a glucometer to confirm the induction of diabetes. Rats with blood glucose level higher than 250 mg/dL were considered as diabetic. Determination of glucose was carried out using a glucometer on day zero (D0) before induction of diabetes, 7 days after induction of diabetes (D7), and 42 days after treatment of the rats with metformin or curcumin on the day 49 (D49).

3.2. Scheduled treatment

Seven days after induction of diabetes, two groups of animals (N+MET300 and D+MET300) were treated with 300 mg/kg bw of metformin. Rats from the N+MET500 and D+MET500 groups received 500 mg/kg bw of metformin. Two groups (N+Cur50 and D+Cur50) were treated by 50 mg/kg bw of curcumin, and finally, rats from the group N+Cur150 and group D+Cur150 received 150 mg/kg bw of curcumin. Metformin and curcumin (Sigma-Aldrich, Ontario-Canada) were dissolved and suspended in deionized water and orally were administrated (gavages) at 10 AM each day for 42 days.

3.3. Sample collection

To assess kidney function, animals were housed in metabolic cage on the day-0, day-7 and day-49 for 8 hours (8 AM to 4 PM) with unlimited access to food and water. Urine samples were collected and urine volume was measured. Urine samples were then acidified with 1 ml of 0.1 M HCl, centrifuged at 12000 rpm for 15 minutes and supernatant was collected and stored at -20°C.

At the end of the day 49, animals were anesthetized using ether and sacrificed. Blood samples were collected from jugular vein, serum was separated and stored at -20°C. The left kidney of each animal was separated, cut into small pieces, and stored at -80°C.

3.4. Analysis of serum and urinary parameters

Fasting blood glucose (FBS) was assessed by a glucometer on day 0, 7, and 49 of the study. Serum creatinine was determined on day 49 whereas urinary urea and creatinine levels were measured on day 0, 7, and 49 of the study. Commercial Pars Azmun colorimetric kits (Pars Azmun, Tehran-Iran) were used to determine serum and urinary creatinine while urine protein was assessed according to the Bradford method (20). Creatinine clearance as an estimate of glomerular filtration rate (GFR) was calculated based on the 8h collected urinary samples according to the following formula and expressed as mL/min: Creatinine clearance=$[(U_V \times U_{Cr})/(S_{Cr} \times 480)]$ where U_V is urine volume (8 hours) and U_{Cr} and S_{Cr} are urinary and serum creatinine, respectively.

3.5. Assessment of oxidative status in kidney tissues

Kidneys were dissected and rinsed with ice-cold saline and completely crushed in liquid nitrogen. The homogenate was suspended in ice-cold lysis buffer [10 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 0.1% triton X100, 0.5 mM Dithiothreitol, protease inhibitor cocktail, pH 7.9] and incubated on ice for 20 minutes. Subsequently, the kidney homogenates were centrifuged at 20000×g for 15 minutes (4°C). The supernatants were collected and stored at -20°C for further analysis. Total protein concentration in tissue homogenates was assessed by Bradford assay using bovine serum albumin (BSA) as standard (20).

Total antioxidant capacity (TAC) in kidney tissues was determined using ferric reducing antioxidant power assay (FRAP) and expressed as nmol/mg of protein (21). Total oxidant status (TOS) was assessed by the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium followed by the quantification of the ferric ion by xylenol orange; the results were expressed as µmol/mg of protein (22). Malondialdehyde (MDA), as an indirect marker for lipid peroxidation, was determined by a spectrofluorometric method and was expressed as µmol/mg of protein (23).

3.6. Determination of antioxidant enzymes activities in kidney tissues

The activity of GPx in kidney homogenate was measured as previously described (24) whereas the activity of SOD was determined based on the amounts of enzyme required to inhibit auto-oxidation of pyrogallol (25) and on the other hand, catalase activity was determined based on Hadwan method(26). The activity of all three enzymes was expressed as U/mg of protein.

3.7. Histopathological examination

For histopathological studies, kidney samples were fixed in 10% neutral-buffered formalin at room temperature for 2 days, processed routinely, embedded in paraffin, sectioned at 5 µm thickness, stained with hematoxylin and eosin, and studied with a routine light microscope.

3.8. Ethical issues

This project was approved by ethics committee and general care of the experimental animals used for this study was done in compliance with the Animal Welfare Act (http://www.nap.edu/openbook.php?record_id=5140&page=114) of Ahvaz Jundishapur University of Medical Sciences. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (#IR.UMSHA.REC.1395.80).

3.9. Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences version 16 (SPSS Inc., Chicago-USA). Values were presented as mean ± SD and statistical significance was defined as *P* values less than 0.05. The one-way ANOVA with post hoc Tukey test was used for comparison between groups.

4. Results

4.1. Effects of metformin and curcumin on blood glucose levels

As shown in Table 1, there was no statistically significant difference ($P=0.183$) in glucose level between the groups at baseline (day 0). Seven days after induction of diabetes, glucose levels in diabetic groups significantly increased compared with the normal group ($P<0.001$). However, data from day 49 (the end of the treatment period) showed that treatment with different doses of metformin did not affect blood glucose levels whereas, treatment with curcumin (150 mg/kg bw) significantly reduced glucose levels ($P<0.001$) compared to the untreated diabetic group.

Table 1. Effects of different doses of metformin or curcmin on fasting blood glucose (mg/dL) in experimental groups

Day	Normal	N+MET300	N+MET500	N+Cur50	N+Cur150	Diabetic	D+MET300	D+MET500	D+Cur50	D+Cur150
D0	88.3±6.9	87.1±10.7	90.5±3.2	91.1±7.4	99.1±7.7	96.8±6.9	96.1±9.7	96.8±5.0	91.0±10.0	94.8±10.0
D7	100.2±5.4[a,b,c,d,e]	100.2±5.3	95.5±7.0	91.1±13.7	78.5±8.6	382.6±33.1[a]	397.0±37.5[b]	386.2±27[c]	393.8±35.2[d]	399.9±31.8[e]
D49	92.3±7.5[a,b,c,d,e]	94.8±8.2	93.5±6.5	96.0±9.0	93.6±6.5	586.6±16.1[a,f]	590.5±12.7[b]	588.6±11.9[c]	570.5±21.0[d]	541.6±27.7[e,f]

Data are represented as mean ± SD. D0 (pre-diabetes), D7 (7 days after induction of diabetes), D49 (42 days treatment with metformin or curcumin after induction of diabetes). In each row, groups with similar alphabetic letters represent significant difference ($P < 0.05$). For simplicity, only differences among main groups have been shown.

4.2. Effect of metformin and curcumin on serum and urinary parameters

Serum creatinine significantly increased in untreated diabetic rats and slightly reduced after treatment with either curcumin or metformin. However, it did not reach statistical significance (Table 2). Urine volume and urinary urea, creatinine and protein levels were measured at day 0 (pre-diabetic), day 7 (7 days after induction of T1DM and before starting of treatment), and day 49 (after 42 days treatment with metformin or curcumin). As shown in Table 2, there were no significant differences in urinary parameters between different groups on D0. Seven days after induction of diabetes, urine volume, urine creatinine, urine urea and urine protein significantly increased in diabetic groups compared to the normal rats. At the end of the experiment on D49, urine volume and urinary urea and protein were found significantly elevated in untreated diabetic group compared with normal group ($P<0.001$) and treatment with either metformin or curcumin caused a significant decrease of these parameters in diabetic treated groups compared to diabetic untreated group ($P<0.001$). However, neither metformin nor curcumin could attenuate these factors in treated diabetic groups to the normal levels. Induction of diabetes markedly enhanced urinary creatinine excretion while the alteration was mostly ameliorated after treatment of rats with metformin or curcumin (except metformin at 500 mg/kg bw) but did not completely returned to the normal level observed in control rats. Creatinine clearance as a marker for GFR was almost tripled in untreated diabetic rats but remarkably reduced after treatment (Table 2).

4.3. Effect of metformin and curcumin on oxidative status in kidneys

The effects of metformin and curcumin on TAC, TOS, and MDA levels in kidney tissues were investigated. As the results are detailed in Table 3, no significant difference was observed in TAC level between groups whereas TOS level was significantly higher in untreated diabetic group than that of the normal group ($P<0.001$). Interestingly, treatment with different doses of either metformin or curcumin resulted in significantly lower levels of TOS compared to the untreated diabetic group.

As shown in Table 3, the induction of diabetes caused a marked increase in MDA levels in untreated diabetic rats ($P=0.013$), the MDA level was significantly ($P=0.012$) declined after treatment of the rats with curcumin (150 mg/kg bw). In the same way, the reduced SOD activity in kidney tissues ($P<0.001$) as a result of STZ-induced diabetes (diabetic rats), was almost completely abrogated ($P=0.003$) by the treatment of the rats with 150 mg/kg bw concentration of curcumin (Figure 1A) and SOD activity restored to the level observed in control rats ($P<0.05$).

Figure 1B shows the effect of different doses of metformin and curcumin on GPx activity in kidney tissues. The GPx activity significantly reduced ($P<0.001$) in untreated diabetic rats, while the treatment of the animals with 150 mg/kg bw of curcumin improved the GPx activity compared to untreated diabetic rat ($P<0.001$) and mostly brought it back to the normal level, although GPx activity statistically did not reach to that of healthy control group. In contrast to the GPx activity, treatment of diabetic rats with 150 mg/kg bw concentration of curcumin (Figure 1C) completely ameliorated diabetes-induced reduction in catalase activity ($P=0.011$) and significantly fostered this enzyme activity to the normal value of control rats.

4.4. Histopathological analysis

On microscopic examination, no pathological changes were observed in the kidney tissues of normal control rats and normal rats treated with different doses of metformin and curcumin, as shown in Figure 2A. The main histopathologic findings in the kidneys of untreated diabetic rats (diabetic group) were glomerular mesangial expansion, thickening of the Bowman capsules, moderate tubular necrosis with mild degenerative and necrotic changes in the glomerular epithelium (Figure 2B), multifocal mononuclear cell infiltration in the interstitial tissue, and diffused interstitial and glomerular hemorrhages (Figure 2C). There was no evidence of advanced lesions such as severe mesangial expansion, nodular sclerosis, glomerulosclerosis, arteriolar hyalinosis and interstitial fibrosis, so the lesions found in this experiment were diagnosed as primary DN.

The diabetic groups that were treated with different doses of metformin and curcumin (D+MET300, D+MET500, D+Cur50 and D+Cur150) showed some features of regeneration, so that necrotic and degenerative changes in the tubular and glomerular epithelium and glomerular/interstitial hemorrhages were reduced. In addition, tubular epithelial hypertrophy and inflammatory cells infiltration were considerably lower in both curcumin and metformin treated diabetic rats compared with untreated diabetic group (Figures 2D-2F). The thicknesses of Bowman capsules were normal and comparable to those of the control rats. The most therapeutic effects were observed with the administration of high dose of curcumin in comparison to other treatments.

5. Discussion

DN is one of the leading causes of end-stage renal disease worldwide and is becoming more prevalent due to the rise in the incidence of obesity and type 2 diabetes. A convincing line of evidence demonstrated the role of oxidative stress, induced by hyperglycemia, in initiation and progression of DN (1,2). Accordingly, different chemical and natural agents have been investigated for their ameliorating effects on oxidative stress and attenuating potency on the progression of DN. Beneficial effects of metformin have previously been shown on diabetes (7,18), however there are still a number of studies questioning its protective efficiency against DN (27,28). On the other hand, the growing interest in the use of natural antioxidant compounds in different diseases is rising rapidly (29-33). Curcumin is mostly known as

Table 2. Effects of different doses of metformin or curcumin on serum and urine parameters in experimental groups

Parameters	Day	Normal	N+MET300	N+MET500	N+Cur50	N+Cur150	Diabetic	D+MET300	D+MET500	D+Cur50	D+Cur150
Serum creatinine (mg/dL)	D49	0.79±0.05[a]	0.79±0.03	0.78±0.04	0.79±0.07	0.82±0.07	0.93±0.04[a]	0.89±0.08	0.86±0.07	0.86±0.06	0.87±0.04
Urine volume (mL/8 h)	D0	1.7±0.3	2.3±1.8	3.2±1.2	1.6±0.8	3.2±16	2.6±0.8	1.7±0.8	2.7±1.0	1.6±0.9	2.1±1.0
	D7	2.5±0.9[a,b,c,d,e]	2.2±0.7	2.2±0.9	1.3±0.3	1.6±0.9	33.5±5.0[a]	26.3±8.5[b]	35.8±9.7[c]	35.8±5.9[d]	33.4±7.1[e]
	D49	2.9±.5[a,b,c,d,e]	4.9±1.5	2.6±1.4	2.9±1.4	2.9±1.4	36.3±2.5[a,f,g,h,i]	22.6±7.0[b,f]	25.6±9.6[c,g]	17.2±4.7[d,h]	23.0±8.1[e,i]
Urine creatinine (mg/8 h)	D0	0.6±.1	0.7±0.3	0.7±.2	0.3±0.2	0.8±0.2	0.9±0.4	0.5±0.3	1.0±0.4	0.5±0.3	1.0±0.6
	D7	0.8±0.3[a,b,c,d,e]	0.7±0.2	0.8±0.4	0.4±0.1	0.5±0.2	3.9±0.9[a]	4.6±2.8[b]	4.4±0.8[c]	3.3±0.8[d]	3.9±0.2[e]
	D49	1.2±0.4[a,b,c,d]	1.1±0.4	0.8±0.5	0.9±0.4	1.0±0.4	4.1±0.4[a,e,f,g]	2.6±0.6[b,e]	3.3±0.8[c]	2.2±0.3[f]	3.0±0.7[d,g]
Urine Urea (mg/8 h)	D0	31.3±14.8	46.55±28.8	45.99±23.7	24.2±11.1	47.0±23.0	46.0±25.1	28.7±18.6	62.9±16.4	28.5±22.2	56.1±31.3
	D9	46.1±21.6[a,b,c,d,e]	54.6±19.1	39.2±18.5	26.4±11.7	33.8±13.9	317.6±62.0[a]	353.2±170.8[b]	382.2±109.4[c]	326.4±74.8[d]	324.3±29.9[e]
	D49	54.4±13.9[a,b,c,d,e]	63.0±25.3	49.6±37.3	51.8±26.4	59.9±5.2	326.6±22.3[a,f,g,h,i]	193.1±54.2[b,f]	222.3±73.2[c,g]	164.1±25.0[d,h]	212.5±62.6[e,i]
Urine protein (mg/8h)	D0	0.3±0.3	0.3±0.2	0.5±0.4	0.4±0.2	0.5±0.2	0.3±0.1	0.3±0.2	0.6±0.2	0.2±0.2	0.4±0.3
	D7	0.3±0.2[a,b,c,d,e]	0.3±0.2	0.3±0.07	0.2±0.04	0.2±0.1	8.6±1.9[a]	7.2±2.1[b]	8.9±2.9[c]	5.6±2.5[d]	9.3±1.0[e]
	D49	0.4±0.2[a,b,c,d,e]	0.7±0.1	0.6±0.5	0.6±0.4	0.6±0.4	8.1±0.8[a,f,g,h,i]	5.4±2[b,f]	5.8±1.3[c,g]	4.1±1.2[d,h]	5.1±1.3[e,i]
Creatinine clearance (ml/min)	D49	0.03±0.01[a,b,c,d]	0.03±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.09±0.01[a,e,f,g]	0.06±0.01[b,e]	0.08±0.02[c]	0.054±0.01[f]	0.06±0.01[d,,g]

Data are represented as mean ± SD. D0 (pre-diabetes), D7 (7 days after induction of diabetes), D49 (after 42 days treatment with metformin or curcumin). In each row, groups with similar alphabetic letters, represent significant difference ($P < 0.05$). For simplicity differences among main groups have been shown, only.

Table 3. Effects of metformin or curcumin on oxidants and antioxidant parameters in kidney tissues of rats.

	Normal	N+MET300	N+MET500	N+Cur50	N0+Cur150	Diabetes	D+MET300	D+MET500	D+Cur50	D+Cur150
TAC (nmol/mg of protein)	281.0±37.5	258.8±61.5	243.3±38.7	271.7±68.4	236.5±52.5	191.4±18.3	207.9±54.8	252.6±16.4	282.1±62.7	251.4±52.8
TOS (µmol/mg of protein)	0.8±0.6[a]	0.8±0.4	0.8±0.5	0.7±0.4	0.7±0.4	3.1±0.6[a,b,c,d,e]	1.0±0.5[b]	1.3±0.6 [c]	1.4±0.8[d]	0.6±0.2[e]
MDA (µmol/mg of protein)	3.4±0.4[a]	3.2±0.5	3.1±0.5	3.5±0.3	3.3±0.5	4.8±0.4[a,b]	3.8±0.8	3.7±0.1	3.8±0.7	3.4±0.5[b]

Data are represented as mean ± SD. In each row, groups with similar alphabetic letters, represent significant difference ($P < 0.05$). For simplicity differences among main groups have been shown, only.

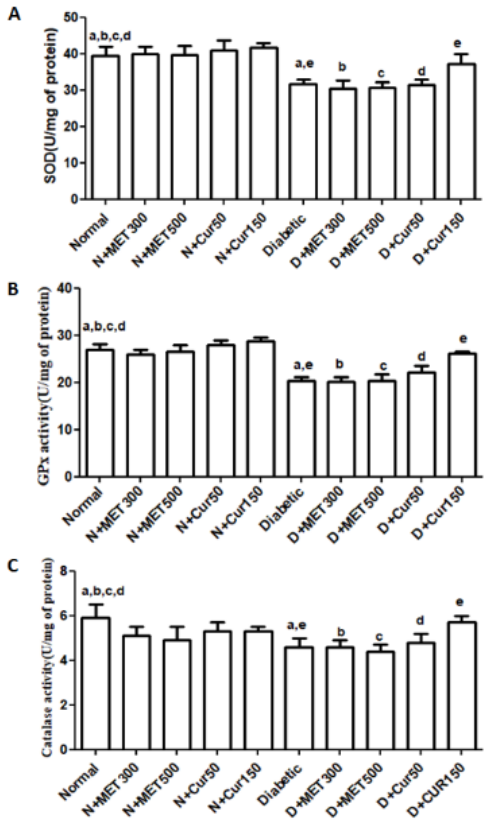

Figure 1. Effects of metformin and curcumin on the activity of antioxidant defence enzymes (A) superoxide dismutase, (B) glutathione peroxidas, and (C) catalase in kidney tissues. The activities of SOD, GPx, and catalase were significantly reduced in diabetic rats but were almost completely ameliorated to the normal valued after the administration of curcumin (150 mg/kg bw). Data are represented as mean ± SD. Columns (groups) with similar alphabetic letter (a, b, c, d, and e) represent significant difference ($P < 0.05$) between groups. For simplicity, differences among main groups have been shown, only.

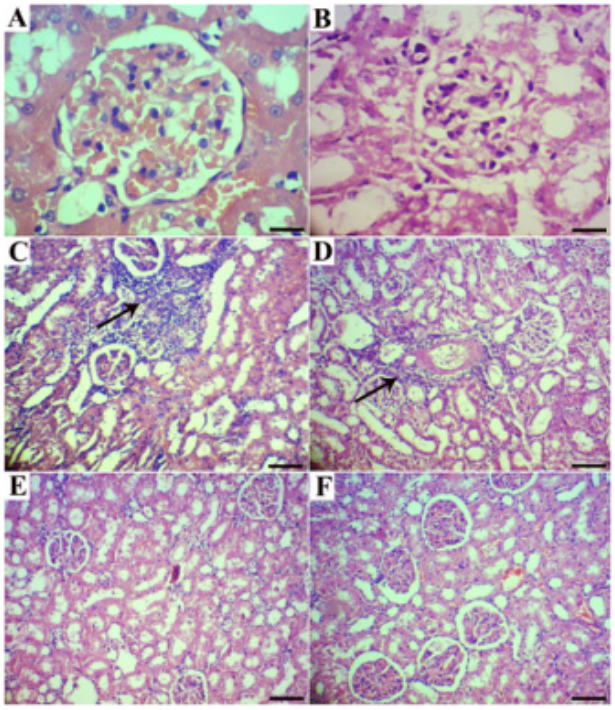

Figure 2. Histological sections of kidney from the normal, untreated and treated diabetic rats. (A) Normal rat. Normal renal histoarchitecture with well-organized distal and proximal convoluted tubules and normal glomerulus and Bowman's capsule (H&E; Bar=40 µm); (B) Diabetic rat. Degenerative and necrotic changes in the tubular and glomerular epithelium and glomerular hypertrophy (H&E; Bar=40 µm); (C) Diabetic rat. Severe infiltration of mononuclear cell in the interstitial tissue (arrow) (H&E; Bar=150 µm); (D) Treated diabetic rat with metformin (500 mg/kg). Mild infiltration of mononuclear cell (arrow) in the interstitial tissue (H&E; Bar=150 µm); (E) Treated diabetic rat with metformin (500 mg/kg). Mild tubular hypertrophy with minimal degenerative changes (H&E; Bar=150 µm); (F) Treated diabetic rat with curcumin (150 mg/kg). Mild glomerular hypertrophy with normal structure (H&E; Bar=150 µm).

an antioxidant agent against oxidative stress induced by hyperglycemia (19,33,34). However, understanding the mechanism of action of curcumin is rudimentary and limited. Due to the importance of oxidative stress in DN, we aimed to investigate the effects of different doses of metformin (300 or 500 mg/kg bw) and curcumin (50 or 150 mg/kg bw) on traditional markers of DN (urinary urea, creatinine and protein) and markers of oxidative stress (TAC, TOS, MDA and SOD, GPX and catalase activity) in kidney tissue of type 1 diabetic rats.

Our results showed that metformin did not decrease blood glucose in treated diabetic rats. Curcumin was not also effective enough to attenuate FBS to the normal level; although, slightly decreased FBS in treated rats and reduced blood glucose in a dose-depended manner which is consistent with the results of previous studies (19,35,36). The low efficiency of metformin (and curcumin) in lowering blood glucose is not a surprising observation. Type-1 diabetic rats with almost no insulin secretion were used in this study whereas metformin is the recommended first-line oral glucose-lowering agent for type-2 diabetes mellitus (T2DM) acting by reducing insulin resistance and improving tissue insulin sensitivity (37,38) and curcumin has insulin sensitizing effects through increasing expression of the insulin receptors (36).

Urine output, proteinuria, and urinary creatinine and urea levels were significantly enhanced in diabetic rats whereas treatment with metformin or curcumin attenuated urine output, decreased proteinuria, and declined urinary creatinine and urea. Urine volume increased over 12-fold in diabetic group and treatment with metformin showed 38% protective activity compared to 52% reduction in urine volume caused by curcumin. Similarly, an over 3-fold increase in urine creatinine in diabetic rats was strongly (46%) prevented by curcumin compared with 36% reduction by metformin. Additionally, curcumin was more effective than metformin in attenuation of the increased urinary urea and protein, as observed in the present study. Although the protective effect of curcumin on DN has previously been reported by Sharma and colleagues (19),

here we showed for the first time the comparative effects of curcumin and metformin on the oxidative status and parameters of DN.

DN is characterized by an eventual decline in GFR, although diabetes in early stages is heralded by glomerular hyperfiltration and an increase in GFR (39). In the present study induction of diabetes induced glomerular hyperfiltration which could be considered as a sign of early phenotype of DN. The observed hyperfiltration is likely due to the structural changes such as cell growth, mesangial expansion, and glomerular basement membrane thickening (40), the characteristics that were later observed in our histopathological examination indicating the presence of early stage of DN in untreated diabetic rats. This finding is in line with previous studies since the presence of glomerular hyperfiltration in incipient DN have previously been reported in both clinical trials and animal models (41,42).

The advantages of curcumin to metformin in improving diabetes-induced alterations were also observed in TAC, TOS, and MDA. Although the reduced TAC and increased TOS in diabetic rats were mainly restored by metformin, curcumin was able to completely normalize TAC, TOS, and MDA to the levels observed in healthy control rats. It has previously been reported that the beneficial anti-diabetic activity of curcumin is probably due to its potent ability to suppress oxidative stress (34), and here, we showed that curcumin is potentially more effective than metformin in restoring antioxidant capacity in kidneys. Therefore, it is postulated that curcumin might be more beneficial agent than metformin in prevention of renal dysfunction in DN. Although the use of metformin has been limited in patients with renal disease because of the perceived risk of lactic acidosis (13), nephroprotective benefits attributed to metformin are hypothesized to be mediated by its potential in mitigating of hyperglycemia-induced oxidative stress in some studies (7,10). Therefore, the clinical use of metformin has been recommended in those with kidney disease (13) however, the results of this study showed that metformin did not sufficiently ameliorate oxidative stress and normalize antioxidant key enzymes activities compared with curcumin.

It is believed that curcumin reduces production of ROS and optimizes oxidative stress leading to the alteration in the activity of SIRT1 and decreasing transcription of NF-kb (15,16). Therefore, it can be concluded that curcumin exhibits its beneficial effects through the abatement of diabetes-induced oxidative stress. Inhibition of the production of ROS, leaves more intact antioxidant enzymes in their reduced form, and thus, enhances TAC of the cells. Interestingly, we showed that SOD, GPx, and catalase activities in kidney tissue which were markedly reduced in diabetic rats were completely recovered by the administration of curcumin (but not metformin). In fact, despite the establishment of the antioxidant property of metformin (7,10) its effect on the activity of SOD, GPx, and catalase has not been investigated so far in DN and to our knowledge, this is the first study comparing metformin with a natural antioxidant agent. Here again, we confirmed the advantages of curcumin to metformin in attenuating of oxidative stress in DN.

Intriguingly, we showed no glucose lowering effect of metformin or curcumin in this study. Therefore, it can be hypothesized that the beneficial effects on improving glomerular hyperfiltration, as observed here, were probably the results of antioxidant properties of metformin or curcumin and not because of their hypoglycemic potency. This finding is of immense importance since the results of previous studies investigating the antioxidant effect of metformin are contentious (7,27,43).

The results of this study may be considered as our incipient understanding about beneficial effects of curcumin in ameliorating DN since a deeper investigation of the nephroprotective mechanisms of curcumin requires determination of ROS as fundamental players of oxidative stress, quantitative measurement of cellular redox reagents such as glutathione which are contributing in the balancing of oxido-redox status, and analysis of SOD, GPx, and catalase at the both gene and protein levels.

6. Conclusions

In conclusion, our data indicated that although both metformin and curcumin could improve traditional biomarkers associated with DN, curcumin was more effective than metformin in reduction of oxidative stress and improving glomerular hyperfiltration, however further detailed studies are required to investigate the effects these compounds in more depth.

Acknowledgments

The authors would like to thank Hamadan University of Medical Sciences for financial support.

Authors' contribution

SA performed all animal and laboratory experiments and MH ran histopathological examination. SA, MTG, and JK prepared the first draft of manuscript. MTG and IK designed the study and IK prepared the final draft of manuscript and supervised the study.

Conflicts of interest

Authors declare no conflict of interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/support

Hamadan University of Medical Sciences supported the study.

References

1. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes Metab Res Rev. 2001;17(3):189-212.
2. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54(6):1615-25. doi: 10.2337/diabetes.54.6.1615.
3. Reutens AT, Atkins RC. Epidemiology of diabetic nephropathy. Contrib Nephrol. 2011;170:1-7. doi: 10.1159/000324934.
4. Van Buren PN, Toto R. Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Adv Chronic Kidney Dis. 2011;18(1):28-41. doi: 10.1053/j.ackd.2010.10.003.
5. Manda G, Checherita AI, Comanescu MV, Hinescu ME. Redox Signaling in diabetic nephropathy: hypertrophy versus death choices in mesangial cells and podocytes. Mediators Inflamm. 2015;2015:604208. doi: 10.1155/2015/604208.
6. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA. 2007;297(8):842-57. doi: 10.1001/jama.297.8.842
7. Alhaider AA, Korashy HM, Sayed-Ahmed MM, Mobark M, Kfoury H, Mansour MA. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through modulation of oxidative stress genes expression. Chem Biol Interact. 2011;192(3):233-42. doi: 10.1016/j.cbi.2011.03.014
8. Hung SC, Chang YK, Liu JS, Kuo KL, Chen YH, Hsu CC et al. Metformin use and mortality in patients with advanced chronic kidney disease: national, retrospective, observational, cohort study. Lancet Diabetes Endocrinol. 2015;3(8):605-14. doi: 10.1016/S2213-8587(15)00123-0.
9. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577-85.
10. Leverve XM, Guigas B, Detaille D, Batandier C, Koceir EA, Chauvin C et al. Mitochondrial metabolism and type-2 diabetes: a specific target of metformin. Diabetes Metab. 2003;29(4):6S88-94. doi: 10.1016/S1262-3636(03)72792-X.
11. Gu J, Ye S, Wang S, Sun W, Hu Y. Metformin inhibits nuclear factor-kappaB activation and inflammatory cytokines expression induced by high glucose via adenosine monophosphate-activated protein kinase activation in rat glomerular mesangial cells in vitro. Chin Med J (Engl). 2014;127(9):1755-60.
12. Song YM, Lee YH, Kim JW, Ham DS, Kang ES, Cha BS et al. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. Autophagy. 2015;11(1):46-59. doi: 10.4161/15548627.2014.984271.
13. Pilmore HL. Review: metformin: potential benefits and use in chronic kidney disease. Nephrology (Carlton. 2010;15(4):412-418. doi: 10.1111/j.1440-1797.2010.01328.x.
14. Soetikno V, Suzuki K, Veeraveedu PT, Arumugam S, Lakshmanan AP, Sone H, et al. Molecular understanding of curcumin in diabetic nephropathy. Drug Discov Today. 2013;18(15-16):756-63. doi: 10.1016/j.drudis.2013.04.009.
15. Zheng H, Whitman SA, Wu W, Wondrak GT, Wong PK, Fang D, et al. Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. Diabetes. 2011;60(11):3055-66. doi: 10.2337/db11-0807.
16. Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I. Regulation of SIRT1 in cellular functions: role of polyphenols. Arch Biochem Biophys. 2010;501(1):79-90. doi: 10.1016/j.abb.2010.05.003.
17. Suresh K. An overview of randomization techniques: An unbiased assessment of outcome in clinical research. J Hum Reprod Sci. 2011;4(1):8-11. doi: 10.4103/0974-1208.82352.
18. Zhai L, Gu J, Yang D, Wang W, Ye S. Metformin ameliorates podocyte damage by restoring renal tissue podocalyxin expression in type 2 diabetic rats. J Diabetes Res. 2015;2015:231825. doi: 10.1155/2015/231825.
19. Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric (Curcuma longa), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol. 2006;33(10):940-5. doi: 10.1111/j.1440-1681.2006.04468.x.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248-54. doi: 10.1016/0003-2697(76)90527-3.
21. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15-27. doi: 10.1016/S0076-6879(99)99005-5.
22. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-1111. doi: 10.1016/j.clinbiochem.2005.08.008.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358. doi: 10.1016/0003-2697(79)90738-3.
24. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1):158-69.
25. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74. doi: 10.1111/j.1432-1033.1974.tb03714.x.
26. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief. 2016;6:194-9. doi: 10.1016/j.dib.2015.12.012
27. Rhee CM, Kovesdy CP, Kalantar-Zadeh K. Risks of metformin in type 2 diabetes and chronic kidney disease: lessons learned from Taiwanese data. Nephron. 2017;135(2):147-53. doi: 10.1159/000450862.
28. Fu J, Fu J, Yuan J, Zhang N, Gao B, Fu G, et al. Anti-diabetic activities of Acanthopanax senticosus polysaccharide (ASP) in combination with metformin. Int J Biol Macromol. 2012;50(3):619-23. doi: 10.1016/j.ijbiomac.2012.01.034.

29. Al-Waili N, Al-Waili H, Al-Waili T, Salom K. Natural antioxidants in the treatment and prevention of diabetic nephropathy; a potential approach that warrants clinical trials. Redox Rep. 2017;22(3):99-118. doi: 10.1080/13510002.2017.1297885.
30. Oshaghi EA, Khodadadi I, Tavilani H, Goodarzi MT. Aqueous extract of *Anethum graveolens* L. has potential antioxidant and antiglycation effects. Iran J Med Sci. 2016;41(4):328-333.
31. Abbasi Oshaghi E, Tavilani H, Khodadadi I, Goodarzi MT. Dill tablet: A potential antioxidant and anti-diabetic medicine. Asian Pacific Journal of Tropical Biomedicine. 2015;5(9):720-7. doi: 10.1016/j.apjtb.2015.06.012.
32. Kim MJ, Lim Y. Protective effect of short-term genistein supplementation on the early stage in diabetes-induced renal damage. Mediators Inflamm. 2013;2013:510212. doi: 10.1155/2013/510212.
33. Kim BH, Lee ES, Choi R, Nawaboot J, Lee MY, Lee EY, et al. Protective effects of curcumin on renal oxidative stress and lipid metabolism in a rat model of type 2 diabetic nephropathy. Yonsei Med J. 2016;57(3):664-73. doi: 10.3349/ymj.2016.57.3.664.
34. Nabavi SF, Thiagarajan R, Rastrelli L, Daglia M, Sobarzo-Sanchez E, Alinezhad H et al. Curcumin: a natural product for diabetes and its complications. Curr Top Med Chem. 2015;15(23):2445-55.
35. Kim T, Davis J, Zhang AJ, He X, Mathews ST. Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. Biochem Biophys Res Commun. 2009;388(2):377-82. doi: 10.1016/j.bbrc.2009.08.018.
36. Xavier S, Sadanandan J, George N, Paulose CS. beta(2)-adrenoceptor and insulin receptor expression in the skeletal muscle of streptozotocin induced diabetic rats: antagonism by vitamin D(3) and curcumin. Eur J Pharmacol. 2012;687(1-3) :14-20. doi: 10.1016/j.ejphar.2012.02.050.
37. Yang X, Xu Z, Zhang C, Cai Z, Zhang J. Metformin, beyond an insulin sensitizer, targeting heart and pancreatic beta cells. Biochim Biophys Acta. 2017;1863(8):1984-90. doi: 10.1016/j.bbadis.2016.09.019.
38. Tan MH, Alquraini H, Mizokami-Stout K, MacEachern M. Metformin: from research to clinical practice. endocrinol Metab Clin North Am. 2016;45(4):819-43. doi: 10.1016/j.ecl.2016.06.008.
39. Magee C, Grieve DJ, Watson CJ, Brazil DP. Diabetic nephropathy: a tangled web to unweave. Cardiovasc Drugs Ther. 2017;31:579-92.
40. Bjornstad P, Roncal C, Milagres T, Pyle L, Lanaspa MA, Bishop FK et al. Hyperfiltration and uricosuria in adolescents with type 1 diabetes. Pediatr Nephrol. 2016;31(5):787-93.
41. Pistrosch F, Herbrig K, Kindel B, Passauer J, Fischer S, Gross P. Rosiglitazone improves glomerular hyperfiltration, renal endothelial dysfunction, and microalbuminuria of incipient diabetic nephropathy in patients. Diabetes. 2005;54(7):2206-11. doi: 10.2337/diabetes.54.7.2206.
42. Zhang C, Meng Y, Liu Q, Xuan M, Zhang L, Deng B et al. Injury to the endothelial surface layer induces glomerular hyperfiltration rats with early-stage diabetes. J Diabetes Res. 2014;2014:953740. doi: 10.1155/2014/953740.
43. Eisenreich A, Leppert U. Update on the Protective Renal Effects of Metformin in Diabetic Nephropathy. Curr Med Chem. 2017;24(31):3397-412. doi: 10.2174/0929867324666170404143102.