Caffeic Acid Modulates miR-636 Expression in Diabetic Nephropathy Rats

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Abstract We investigated the action of caffeic acid in regulating miR-636 expression level in kidney of streptozotocin-induced diabetic rats. Streptozotocin-induced diabetic rats were orally treated with caffeic acid at 40 mg/kg/day for 8 weeks. At the end of the treatment, body and kidney weight and blood glucose levels were determined, blood, urine, and kidneys were collected for biochemical and histological examination. Expression levels of miR-636 were determined in liver by qRT-PCR. Induction of diabetic nephropathy by streptozotocin was evidenced by displayed elevated levels of serum creatinine, blood urea nitrogen, microalbuminuria and urinary albumin/creatinine ratio in addition to renal hypotrophy. Caffeic acid (CA) can ameliorate renal damage and significantly decreased the fasting blood glucose, cholesterol and triglyceride in diabetic rats. CA treatment improved histological architecture in the diabetic kidney. CA significantly down regulate miR-636 expression level in the kidney of diabetic rats in comparison to healthy group. Overall, caffeic acid down regulates miR-636 expression level which is involved in development of diabetic nephropathy and might therefore be potential attractive therapeutic agent to pursue in DN.

Keywords Diabetic nephropathy · Caffeic acid · miRNA

Introduction

Diabetic nephropathy (DN), a severe microvascular complication frequently associated with both type 1 and 2 diabetes mellitus and it is a leading cause of renal failure [1]. Among symptoms of diabetic nephropathy, albuminuria is an early marker, and hence, a major biochemical feature in the clinical diagnosis of diabetic nephropathy [2].

Autophagy is catabolic pathway by which mammalian cells degrade organelles to maintain intracellular homeostasis. The accumulation of damaged organelles is associated with the pathogenesis of diabetic nephropathy. Autophagy in the kidney is activated under some stress conditions, such as oxidative stress and hypoxia in proximal tubular cells, and under normal conditions in podocytes. It was hypothesis that autophagy is involved in the pathogenesis of diabetic nephropathy [3].

Mounting evidence indicates that microRNAs (miRNAs)-mediated post-transcriptional regulation of gene expression represents an integral part of the autophagy regulatory network and may have a substantial effect on autophagy-related physiological and pathological conditions [4]. A substantial number of miRNAs show differential expression in diabetic kidney diseases, and so have been investigated for possible use in diagnosis. Furthermore, there is increasing interest in their use as prognostic markers and determining treatment response [5].

Unraveling the significance of autophagy-miRNA interaction in DKD might lead the way to novel diagnostic and molecular therapeutic targets for diabetic kidney disease [6]. MicroRNA-636 is noted to be one of unique

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genetic cluster that share function related to diabetes mellitus and renal diseases [7].

Although our knowledge of DN is continuously increasing, no treatment strategies specifically target the pathogenesis of DN beyond controlling glucose levels, blood lipid levels, and high blood pressure. As a result, the identification of potential drugs targeting the molecular pathogenesis of DN is critical for improving the prognosis and survival of patients with DN [8].

Polyphenols are the abundant micronutrient in our diet and attention has been given to them for the prevention of degenerative diseases, one of them is caffeic acid. Caffeic acid possesses multiple biological effects, such as antibacterial, antioxidant, anti-inflammatory, anticancer growth, and antidiabetic activities [9]. Caffeic acid phenethyl ester (CAPE), a caffeic acid derivative induced autophagy activities in hepatic stellate cells (HSCs) through accumulating autophagosomes [10].

Previously, many studies directed towards discovering new drugs from natural sources in treating diabetes [11]. At present, only few of the available drugs are effective in the treatment of diabetic nephropathy; one of the diabetes complications; therefore, there is need to search for safe and effective drugs against the condition. This study was conducted due to the important miRNAs in complications of diabetes and given the current evidence regarding the beneficial effects of caffeic acid in patients with diabetes.

We had a bioinformatics analysis to retrieve and choose a miRNA (miR-636) relevant to DKD that regulates autophagy gene based on previous microarray studies. So we aimed to investigate the action mechanism of caffeic acid in autophagy pathway via regulating miR-636 expression level in kidney of streptozotocin-induced diabetic rats.

Materials and Methods

Chemicals

Streptozotocin and caffeic acid were purchased from Sigma Chemical Co, St. Louis, Mo, USA.

Animals

Forty-eight adult male Albino Wistar rats, weighting 190–220 g were purchased from Egyptian Co. for experimental animals Import, Helwan, Egypt. The animals were kept 1 week in an animal house for an acclimatization period prior to the start of the experiment. They were housed 4/cage and received normal basal diet and tap water

ad libitum in a constant environment (room temperature 28 ± 2 °C, room humidity $60 \pm 5\%$) with a 12 h light and 12 h dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee. All rats were maintained according to institutional guidelines. Animal procedures were carried out in compliance with the Institutional Standards for Humane Care and Use of Laboratory Animals.

Experimental Diabetic Nephropathy Model and Treatment Protocol

Diabetic Nephropathy Rats Induction

Diabetes Mellitus was induced in albino rats by a single intraperitoneal injection of streptozotocin (55 mg/kg body weight dissolved in 0.1 M cold citrate buffer; pH 4.5) [12] to overnight fasted rats. After STZ injection, rats had access to food and drink 5% glucose solution for 48 h to counter hypoglycemic shock and to avoid early mortality due to excessive insulin released from damaged pancreatic beta cells [13]. After 3 days, blood samples were collected through the tail vein and blood glucose levels were measured using a glucometer (OneTouch Horizon, LifeScan, Johnson & Johnson, CA, USA). Animals with blood glucose above 200 mg/dl were used for the study. Rats with blood glucose lower than 200 mg/dl were excluded from the study. Diabetic nephropathy were developed after 4 weeks of STZ induction.

Experimental Design

The animals were divided equally into 3 groups. Age-matched control rats ($n = 16$) received equal volume of vehicle (a single tail vein injection of 0.1 mol/l citrate buffer) served as the control group (C). The other rats were intravenously injected with freshly prepared streptozotocin, the study was started 1 week after STZ injection and divided into two random groups:

- Diabetic nephropathy group (N) ($n = 16$): scarified after 4 weeks of STZ injection.
- Diabetic nephropathy treated group (T) ($n = 16$): diabetic nephropathy rats treated with caffeic acid (CA) (40 mg/kg/day) [14] for 2 months [15] then scarified.

At the end of the experiment, animals were sacrificed by cervical dislocation under ether anesthesia. Kidneys were rinsed in saline buffer (0.9%NaCl) and weighed. After weighing, left kidneys were kept in 4% paraformaldehyde solution for histology examination whereas right kidneys were stored at -80 °C for RNA isolation.

Histopathological Examination

Renal tissues, fixed in 10% paraformaldehyde for 1 day at room temperature, dehydrated in a graded series of ethanol, embedded in paraffin, and cut into 4 μm thick sections on a rotary microtome. These sections were stained with hematoxylin–eosin (HE) reagent to assess alterations in the glomerulus and tubules. Renal sections were observed under a light microscope at a 400 $\times$ magnification and photographed [16].

Sampling and Biochemical Analysis

Blood samples of rats collected from optical vein before scarifying were centrifuged at 2000 g for 10 min at 4 $^{\circ}\text{C}$ and aliquot. Creatinine, Blood Urea Nitrogen (BUN), Cholesterol, Triglycerides, LDL and HDL-cholesterol were quantified according to the manufacturers' guidelines (Biomed Diagnostics, Egypt). Urine samples were collected in individual metabolic cages for 24 h, the day before the end of treatment. Sugar, albumin, creatinine and urea levels were estimated using the commercially available colorimetric kits (Biomed Diagnostics, Egypt) according to the instructions of the supplier. In addition, creatinine clearance, albumin/creatinine ratio, were calculated.

Bioinformatic Analysis to Retrieve miRNA Relevant Diabetic Nephropathy Based on Previous Microarray Studies

We have chosen DN-characteristic miRNA-636 based on previous microarray studies such asmiro-Ontology database (available at <http://ferrolab.dmi.unict.it/miro/>), miR-Walk database [17] to perform network visualization analysis and kidney tissue specificity analysis to ensure miRNA deregulation in DN. These databases confirmed the correlation between the selected miRNA over-expression and DN development with higher ranking score. Afterwards, biomarker verification were performed to ensure tissue specificity of miRNA-636 through microRNA. Org Target and expression database (available at <http://www.microrna.org/microrna/home.do>).

Pathways Enrichment Analysis

To infer putative targets of the expressed miRNA-636, we utilized different databases: miro-Ontology database (available at (<http://ferrolab.dmi.unict.it/miro/>)) and DIANA-mirPath software [18].

RNA Extraction and RT-qPCR

Total RNA Isolation from Kidneys Tissues

For gene expression analysis, mRNA was isolated from kidney homogenate using MiRNeasy isolation Kit (QIAGEN, Germany) according to the manufacturer's instructions. RNA quality and concentration were then determined using an Ultraspec 1000, UV/visible spectrophotometer (Amersham Pharmacia Biotech, Cambridge, England) and NanoDrop 2000 (Thermoscientific, USA). All mRNA samples were quantified by spectrophotometry and stored at -80°C until further analysis.

Quantitative Real-Time PCR

For microRNA expression analysis, cDNA was prepared from 1 to 2 μg of total RNA from kidney tissue using miScript RT Kit (Qiagen, Germany) according to manufacturer instructions through reverse transcription PCR. The concentration of cDNA resulted from tissue diluted 2 times by RNase- free water before proceeding further PCR analysis. Relative expression of miR-636 was estimated using miScript SYBR Green PCR Kit (Qiagen, USA) through quantitative Real Time PCR (qPCR) with the manufacturer provided miScript universe primer, miRNA-636 specific forward primer (Catalogue no. MS00033901, Qiagen) based on the miRNA sequence obtained from the miRbase database (<http://microrna.sanger.ac.uk/%29%28>). Data were analyzed following the $2^{-\Delta\text{Ct}}$ method, using SNORD-95 and RNU-6-2 as endogenous control to normalize any input and cDNA conversion efficiency variations.

Data analysis and Statistics

Quantitative data were expressed as mean $\pm$ SEM. Data were analyzed using one analysis of variance (ANOVA) followed by a multiple comparisons testing used to assess differences between individual pairs of means among the groups. p values < 0.05 were considered statistically significant. Fold change in expression by RT-PCR were calculated by $2^{-\Delta\text{Ct}}$ method, where $\Delta\text{Ct} = \text{Ct}_{\text{gene of interest}} - \text{Ct}_{\text{endogenous control}}$. The $2^{-\Delta\text{Ct}}$ values were log transformed for statistical analyses and representation. Pearson correlation test was used for correlation analysis.

Results

Effects of Caffeic Acid on Blood Glucose Level

Administration of STZ led to significant increase (Table 1; $p < 0.001$) in fasting blood glucose levels in the diabetic group as compared to the control group throughout 4 weeks after induction. Furthermore, STZ-diabetic rats treated with CA demonstrated a significant decrease ($p < 0.001$) in the fasting blood glucose levels at the end of 8 weeks of treatment.

Effects of Caffeic Acid on Rat body and Kidney Weight

After induction of nephropathy in diabetic animals, body weight measures were significantly ($p < 0.001$) lowered compared to the control group. However, body weight measures of rats treated with CA for 2 months were significantly ($p < 0.001$) higher compared to DN rats (Table 1).

The kidney weight in diabetic rats were significantly increased as compared to those in the normal control group. Treatment of STZ-diabetic rats with CA ameliorated the weight of kidney approaching the weights of normal control group.

Effects of Caffeic Acid on Renal Function

To explore the effects of CA treatment on renal function, the levels of BUN, urine creatinine, serum creatinine, creatinine clearance, microalbuminuria and UACR were investigated in STZ-induced diabetic rats (Table 2).

The STZ-diabetic rats showed a significant decrease in the level of creatinine in urine with a significant increase in serum as compared to the normal control group. After treatment with CA, the creatinine content increased significantly in urine with a significant decrease in serum.

A significantly increased level of UACR ($p < 0.001$) was evident in the N group compared to the C group. The treatment of the diabetic rats with CA significantly recovered the UACR in comparison with the N and C groups ($p < 0.001$, $p < 0.05$ respectively), suggesting that the CA attenuated the progression of the DN.

Likewise, the BUN levels in diabetic rats were significantly higher than normal rats ($p < 0.001$). Whereas treatment with CA significantly decreased this level.

The urine volume and microalbuminuria level in the diabetic rats were significantly ($p < 0.001$) increased when compared to normal control group. While diabetic rats treated with CA showed significant ($p < 0.001$) decrease in urine volume and microalbuminuria levels when compared to diabetic rats.

Effects of Caffeic Acid on Lipid Profile

The results of the serum lipid profile showed that streptozotocin injection led to the development of hyperlipidemia in which serum triglyceride, total cholesterol, and LDL-cholesterol markedly ($p < 0.001$) increased when compared to the control group. However, HDL-cholesterol significantly decreased ($p < 0.001$) in diabetic rats in comparison with the normal control. On the other hand, CA administration led to hypolipidemic effect on serum lipids of diabetic rats as given in Table 3. The levels of TG, TC and LDL-C were significantly reduced as compared with diabetic rats but still significantly higher than controls.

The Effect of Caffeic Acid on Renal Morphological Changes (Kidney Histology)

The histopathological observations of the rat kidneys revealed that the normal control group rats showed normal glomerular size and kidney tubules with healthy epithelial cells (Fig. 1a). According to H&E stained kidney specimens of STZ-diabetic rats exhibited moderate to severe focal inflammatory cells infiltration as well as aggregation, glomerular congestion with periglomerular fibrosis and

Table 1 Effect of caffeic acid on blood glucose level, body and kidney weight of STZ-diabetic rats

Groups	C	N	T
Body weight (g)	234.34 ± 4.89 ^c	180.00 ± 3.13 ^b	218.75 ± 3.75 ^a
Kidney weight (g)	0.71 ± 0.03 ^b	1.26 ± 0.09 ^b	1.11 ± 0.06 ^d
(kidney weight/Body weight) %	0.30 ± 0.01 ^c	0.7 ± 0.04 ^b	0.51 ± 0.03 ^a
Glucose level (mg/dl)	90.62 ± 1.82 ^c	428.75 ± 19.29 ^b	257.50 ± 4.009 ^c

^aTreatment (T) group was compared with diabetic nephropathy (N) group, $p < 0.001$

^bDiabetic nephropathy group was compared with control (C), $p < 0.001$

^cTreatment group was compared with control, $p < 0.05$

^dTreatment group was compared with diabetic nephropathy group, $p < 0.05$

^eTreatment group was compared with control, $p < 0.001$

Table 2 Effect of caffeic acid on kidney function parameters

Groups	C	N	T
BUN (mg/dl)	14.89 ± 0.14 ^b	36.36 ± 0.89 ^c	21.64 ± 0.39 ^a
Scr (mg/dl)	1.47 ± 0.04 ^b	2.53 ± 0.04 ^c	1.85 ± 0.02 ^a
Ucr (mg/dl)	55.83 ± 0.56 ^c	35.38 ± 0.38 ^b	38.30 ± 0.28 ^d
Ccr (ml/min.)	0.19 ± 0.01 ^{NS}	0.16 ± 0.001 ^c	0.19 ± 0.004 ^a
Microalbuminuria (mg/dl)	16.05 ± 0.16 ^b	33.813 ± 0.91 ^c	21.86 ± 0.51 ^a
UACR (mg/g)	288.08 ± 4.85 ^b	956.42 ± 26.51 ^c	571.45 ± 16.47 ^a
Urine volume (ml)	7.22 ± 0.12 ^c	16.44 ± 0.29 ^c	13.19 ± 0.25 ^a

^aTreatment group was compared with diabetic nephropathy group, $p < 0.001$ ^bTreatment group was compared with control, $p < 0.05$ ^cDiabetic nephropathy group was compared with normal control, $p < 0.001$ ^dTreatment group was compared with diabetic nephropathy group, $p < 0.01$ ^eTreatment group was compared with control, $p < 0.001$ using one-way ANOVA with LSD test. Data presented as mean ± SE^{NS}When $p > 0.05$ **Table 3** Effect of caffeic acid on lipid profile parameters

Groups	C	N	T
Triglycerides (mg/dl)	80.82 ± 0.57 ^d	179.64 ± 5.25 ^c	89.06 ± 0.454 ^a
Total cholesterol (mg/dl)	72.66 ± 0.58 ^b	124.77 ± 2.98 ^c	85.20 ± 0.67 ^a
HDL-cholesterol (mg/dl)	36.27 ± 2.24 ^b	16.69 ± 0.48 ^c	31.50 ± 0.35 ^a
LDL-cholesterol (mg/dl)	20.22 ± 0.72 ^b	72.16 ± 2.46 ^c	35.89 ± 0.42 ^a

^aTreatment group was compared with diabetic nephropathy group, $p < 0.001$ ^bTreatment group was compared with control, $p < 0.001$ ^cDiabetic nephropathy group was compared with normal control, $p < 0.001$ ^dTreatment group was compared with control, $p < 0.01$ using one-way ANOVA with LSD test. Data presented as mean ± SE **Fig. 1** Photomicrographs of kidney (Hematoxylin and Eosin staining under a light microscope at 400× magnification). **a** Control rats showing no abnormalities; **b** diabetic nephropathy rats showing Focal inflammatory cells infiltration, glomerular congestion with

focal hemorrhage (Fig. 1b). Treatment with CA for 2 months significantly ameliorated these changes in the kidneys and reveals features similar to normal (Fig. 1c).

periglomerular fibrosis and focal hemorrhage; **c** Caffeic acid treated group showing decreased glomerular congestion, swelling of tubular lining epithelium and focal aggregation of glomeruli, *T* tubules, *GS* glomerular space, *VD* vacuolar degeneration

The Effect of Caffeic acid on miRNA-636 Expression

We examined the effect of diabetes on the regulation of miRNA-636 in kidney tissues by qPCR. Surprisingly, we

found significantly higher levels of miRNA-636 in kidney tissue from DN rats relative to control group ($p < 0.01$). However, the caffeic acid treatment significantly reduced the level of renal miR-636 expression ($p < 0.001$) (Fig. 2).

Discussion

Drug-induced diabetes is one of the most commonly used experimental diabetic models [11]. Streptozotocin is widely employed to induce experimental DN, as it is selectively destroying the islets of Langerhans [19]. So through the approaches of using STZ induced DN in rats, we investigated the modulatory effects of CA on the expression of miRNA-636 involved in pathogenesis of DN. Our study provides the first evidence that CA attenuates DN by modulating miRNA-636 expression which could be a regulator of autophagy gene.

Albuminuria is a dominant feature of developing DN and all therapeutic interventions inducing a reduction of the albumin excretion rate have a protective effect on renal function [2].

DN is characterized by pathophysiological changes in glomerular hyperfiltration, renal hypertrophy, tubular function and then progress to proteinuria and reduction of glomerular filtration rate [20]. Hypoalbuminemia is considered as a strongest predictor of death in patients with renal failure. Albumin is by far the most abundant protein in nephrotic urine [21]. In our STZ-diabetic rats, albumin was increased significantly, thus demonstrating that

albuminuria was related to deteriorating kidney function. Treatment with CA normalized these levels thus, exhibiting its beneficial role against microalbuminuria.

Much of the diabetes research suggests that hyperglycemia is the inducer of diabetic complications, including nephropathy [8]. In our study, administration of STZ led to significant increase ($p < 0.001$) in blood glucose level which was lowered on treatment with CA thus confirming the antihyperglycemic activity of the CA as reported by earlier established studies [13]. Currently, CA has been widely investigated to have antidiabetic activity [10].

It can be postulated that kidney damage in diabetes might be triggered by hyperglycemia, which subsequently leads to perturbations in autophagy and associated signaling [22]. Caffeic acid exerts anti-degenerative effects and promotes the survival of islets in animal model. In the study conducted by Jung et al. [23], in mice, caffeic acid has been found to preserve islet normal histological appearance against the control which exhibited islet boundary definition loss and degeneration.

Histopathological examination of kidney sections of STZ diabetic rats showed severe vacuolar degeneration of tubules, increased glomerular space and basement membrane thickening. Treatment with CA significantly reduced the aforementioned alterations, thus demonstrating protective role in renal damage.

STZ-induced diabetes is associated with significant reduction in the body weight due to hyperglycemia, increased muscle wasting and loss of tissue proteins [24]. In the current study, the body weight of STZ-diabetic rats was progressively reduced and treatment with CA improved the body weight significantly, thus indicating prevention of muscle tissue damage caused due to hyperglycemia.

Increase in the weight of kidney (hypertrophy) in proportion to the body weight is observed in STZ-induced rats. Local alterations in the production of one or more growth factors such as over expression of transforming growth factor beta 1 in the kidney especially in proximal convoluted tubules cells and glomerular mesangial cells are proposed in the development of renal hypertrophy. An increase in the rate of protein synthesis and/or decrease in the degradation of renal extracellular components might also lead to renal hypertrophy [24]. CA treatment reduced kidney/body weight ratio, thus demonstrating reversal of kidney hypertrophy in STZ-diabetic rats.

Increased serum creatinine level and BUN along with decreased excretion of creatinine in the urine are indicators of the development of diabetic nephropathy [25]. Reversal of these effects was observed in STZ-diabetic rats treated with CA. These findings suggest that CA can effectively ameliorate renal damage in diabetic rats.

Fig. 2 Expression of miRNA-636 in the kidney of diabetic rats. * $p < 0.05$ compared to control group, ** $p < 0.01$ compared to diabetic nephropathy group, *** $p < 0.001$ compared with the treatment group. Statistical analysis was performed using ANOVA. Data are presented as mean $\pm$ S.E

Diabetes mellitus (DM) leads to fatty liver, hypercholesterolemia and hypertriglyceridemia. Moreover, elevated cholesterol levels are associated with diabetic nephropathy [26]. These increased levels were reversed towards normal after treatment with CA, thus demonstrating its potential to improve lipid metabolism.

Recent studies provided a perspective on the involvement of autophagy in the pathogenesis of diabetic nephropathy and whether it can be a new therapeutic target to treat this condition. Future studies will provide clear evidence to determine whether autophagy should be considered a novel therapeutic target for diabetic nephropathy [4].

Chuang et al. [27] suggested that a caffeic acid derivative attenuates renal injury through both reducing extracellular matrix synthesis and elevating extracellular matrix degradation. It also inhibits renal fibrosis by blocking the TGF- β /Smad signaling pathway.

Caffeic acid is evolving as potential therapeutic agent which prevents and treats diabetic complications based on its multiple targeting actions against diabetes [9]. Dysregulation of some miRNAs has been shown in diabetic or renal diseases [6].

We applied bioinformatics analysis to retrieve miRNA related to DN. Accordingly, Eissa et al. [7] highlights the possibility of using miR-636 as a pharmacological target for diabetic kidney diseases in T2DM.

MiR-636 was differentially expressed in type 1 DM and gestational diabetes mellitus [6]. In silico data analysis for the predicted targets of miRNA-636 highlights its involvement in pathways related to fatty acid metabolisms, glucose homeostasis, and renal fibrosis [7].

Because miRNAs are linked to regulation of autophagy pathway and autophagy involves a series of dynamic membrane rearrangements controlled by a set of ATG proteins [6]. So by using bioinformatics analysis and searching online for “miRNA targets” using Pictar, TargetScan, and MiRanda and through a function and pathway analysis using the KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) databases, we found that miR-636 targets Microtubule-associated proteins 1A/1B light chain 3C (MAP1LC3C) mRNA which is an autophagy gene. MAP1LC3C are a specific residual protein needed for the fusion activity are essential for the proteins role in autophagosome biogenesis [28].

The expression levels of miRNA-636 was significantly up-regulated in the kidneys of STZ-rats and inhibited by caffeic acid treatment, which suggests that this miRNA could be new therapeutic targets for DN.

We demonstrated an increase in miR-636 expression level of renal tissues in diabetic rats during the progression of diabetes. However, this rise was reduced in rats treated with CA which suggest its involvement in autophagy

induction with curative properties. Vijayakurup et al. [29] found that Phenethyl caffeate benzoxanthene lignan (PCBL), a semisynthetic compound derived from caffeic acid phenethyl ester (CAPE), induces autophagy. Additional study has explored the role of caffeic acid derivatives in attenuation of autophagy in cancer [30]. A previous study by the authors revealed that treatment with caffeic acid suppressed the expression of some other miRNAs with subsequent induction of autophagy in HFD-STZ -induced diabetic rats [6].

Several studies reported the compromised autophagy in DN. The kidney cells seem to be more susceptible to the metabolic alterations due to high level of basal autophagy [3]. Thus, deficiency of autophagy might play a critical role in the progression of diabetes-associated renal damages [6].

Our rats' model revealed that treatment with caffeic acid suppressed the expression of miRNA-636 with subsequent induction of autophagy which ameliorated glomerular changes, albuminuria with reducing blood glucose levels in STZ -induced diabetic rats. We hypothesize that caffeic acid seems to trigger mitogen-activated protein kinases (MAPK) signaling via regulation of miR-636 which in turn induces autophagy that ameliorates diabetic nephropathy. Caffeic acid triggered induction of MAPK and the autophagic response in tumor [30].

Conclusion

In summary, “caffeic acid” a new drug from natural sources which exhibits a protective action in STZ-induced diabetic nephropathy rats via down regulating miRNA-636 expression associated with autophagy could be of great concern in countries with limited resources and high incidence of diabetic nephropathy. In conclusion, administration of CA to STZ diabetic rats decreased blood glucose level, normalized the cholesterol and triglycerides level, and ameliorated the serum and urine parameters along with normalization of kidney histopathological status.

Although the results of this study are still preliminary, they provide a proof of concept of the feasibility of such integrative novel approach for therapeutic drug discovery in diabetic kidney diseases.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and all procedures performed in studies involving animals were in accordance with the ethical standards of Institutional Ethical Committee.

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