Silymarin Attenuates ELMO-1 and KIM-1 Expression and Oxidative Stress in the Kidney of Rats with Type 2 Diabetes

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Received: 19 September 2017 / Accepted: 24 January 2018 / Published online: 6 February 2018
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Abstract Chronic diabetes mellitus is accompanied with overexpression of ELMO1 and KIM1 and enhanced oxidative stress. This study was aimed to evaluate the effects of administration of silymarin on oxidative stress markers and ELMO1 and KIM1 expression in the kidney tissue of type 2 diabetic rats. In this experimental study, 36 male Wistar rats were divided into 6 groups: Control, silymarin-treated control (60 and 120 mg/kg/day), diabetic, and silymarin-treated diabetic groups (60 and 120 mg/kg/day). Tissue levels of oxidative stress and biochemical parameters were measured by spectrophotometric methods. Lipid peroxidation levels in the kidney tissue were measured by fluorometric method. Insulin was determined using immunoassay. Gene expression analysis was determined by qPCR technique. The level of expression of ELMO1 and KIM1 in the diabetic groups treated with silymarin was significantly reduced ($P < 0.001$). Total antioxidant levels and thiol groups contents increased ($P < 0.001$) dramatically in treated groups. A significant decrease in tissue levels of malondialdehyde and total oxidant were observed in the silymarin treated diabetic rats ($P < 0.001$). The results showed that the urinary amount of protein in the treatment groups was significantly lower than of diabetic control ($P < 0.001$). These results indicate that silymarin has a blood glucose lowering effect and, due to its antioxidant properties, increases the antioxidant parameters and reduces the oxidant markers. The

administration of silymarin has beneficial effects on kidney of diabetic rats with reduction of ELMO1 and KIM1 expression.

Keywords Silymarin · Diabetes mellitus · Kidney · KIM-1 · ELMO-1 protein · Oxidative stress

Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disorder with insulin resistance, a defect in the secretion of insulin or its receptor disorder, which is associated with deficiencies in the metabolism of lipids, carbohydrates and proteins [1]. Type 2 diabetes is associated with many microvascular and macrovascular complications, including diabetic nephropathy, neuropathy and retinopathy [2]. Many patients with type 2 diabetes mellitus have diabetic nephropathy [3]. The most important symptoms of diabetic nephropathy such as reduce glomerular filtration rate and increase the secretion of urine albumin [4]. Studies have shown that some genes are associated with diabetic nephropathy and kidney disorders. ELMO1 (Engulfment and cell motility 1) and KIM1 (Kidney injury molecule 1) are two very important genes in diabetic nephropathy. Engulfment and cell motility protein has 720 amino acids and this gene in human is located on chromosome 7 and in mice is located on chromosome 13 [5]. ELMO1 has great importance in the development of diabetic nephropathy [6]. ELMO1 has a low expression in tubular and glomerular cells in normal rats, while its expression increases in the diabetic rats [7]. Over expression of the ELMO1 increases the expression of extracellular matrix genes such as transforming growth factor beta

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1 (TGF- β 1) and collagen-1 and increases the glomerular basement membrane diameter [8].

The oxidative stress is a phenomenon caused by an imbalance between the production of free radicals and antioxidant defense of the body and plays very important role in the complications and pathogenesis of diabetes [9, 10]. One of the factors that lead to increased oxidative stress is hyperglycemia [11]. Oxidative stress appears as important factor that leads cells to severe damages and diseases [12]. Oxidative stress and lipid peroxidation due to increased free radicals of oxygen play a key role in the damage of the kidney tissue in diabetic patients [13, 14].

Silymarin is a milk thistle (*Silybum marianum*) extract, a herbal medicine with antioxidant, anti-apoptosis and anti-inflammatory properties [15] which has different flavonolignans such as silybin, isosilybin A, isosilybin B, silychristin and silydianin [16]. Various therapeutic activities of silymarin such as nephro-protective have been shown by different studies [17]. Silymarin is used for treatment of many kidney disorders, including drug-induced toxicity, alloxan-induced diabetes mellitus and renal ischemia-reperfusion injury [18]. Thus, the main aim of this study was to evaluate the kidney-protective effects of silymarin via oxidative stress and KIM1 and ELMO1 expression in the kidney of rats with T2DM.

Materials and Methods

Animals

Thirty six male rats, weighing 200–220 g, were housed at room temperature (22–25 °C) with a relative humidity of 45–55% and 12:12-h light/dark cycles and free access to food and water. All the procedures for the treatment of animals and ethic were approved by the Research Committee of Hamadan University of Medical Sciences, Iran.

Work Design

Type 2 diabetes was induced by injection of (STZ) (60 mg/kg) dissolved in 0.1 M citrate buffer (pH = 4.5), 15 min after the prescription of nicotinamide (110 mg/kg; i.p) in 12-h-fasted rats. Citrate buffer was injected alone in control rats [19]. Afterwards, 7 days blood glucose levels were measured using a strip-operated blood glucose sensor. Animals were considered diabetic if fasting blood glucose levels exceeded 250 mg/dl. The rats were divided into six groups (n = 6 in each group): normal control (group I), normal control treated with silymarin (Goldaru Co. Iran) 60 mg/kg/day (group II), control treated with silymarin 120 mg/kg/day (group III), diabetic (group IV), diabetic treated with silymarin 60 mg/kg/day (group V), diabetic

treated with silymarin 120 mg/kg/day (group VI). Silymarin treatment was performed orally for 60 days. The dosage was regulated every week. At the end of the experimental period, fasted rats were anesthetized with ketamine (50 mg/kg) and blood samples (10 mL) were collected from each rat for biochemical measurements. Thereafter, the rats were scarified and the kidneys were quickly frozen in liquid nitrogen and kept at – 80 °C.

Measurement of Body Weight and Kidney Weight

The weight of the rats was measured on two steps on the first day and the sixth day. After sacrificing rats, removed the kidney tissue and recorded weight them.

Measurement of Fasting Blood Glucose, Insulin and HOMA-IR

Fasting blood glucose level was measured with a glucometer (Accucheck; Roche, Germany). Insulin was measured using the rat Insulin ELISA Kit (Mercodia, Uppsala, Sweden). Homeostasis model assessment for insulin secretion (HOMA-IR) was calculated as follows: $\text{HOMA-IR} = \text{Fasting insulin } (\mu\text{U/ml}) \times \text{Fasting glucose (mg/dl)} / 405$ [20].

Measurement of Urea, Creatinine and Uric Acid and BUN in Serum and 24-h Urine

Blood and urine levels of urea, creatinine, uric acid and BUN were measured using Pars Azmoun kit (Iran) and enzymatic methods.

Collection of 24-h Urine

In this study, urine collection was performed in three steps on the first day, the seventh day, and the sixtieth day. To collect 24-h urine, the rat was first placed in the metabolic cages, and after 24 h the urine sample was collected and urine volume was recorded, and 10 ml of urine was collected into the Falcon tube and was stored at temperature of – 20° C [21].

Tissue Collection and Processing

Kidney tissues from healthy and treated groups of rats were excised and rinsed with ice-cold saline and grounded into a fine powder using liquid nitrogen. The homogenate was resuspended in ice cold lysis buffer (10 mM HEPES, 10 mM KCl, 1.5 mM MgCl₂, 1 mM EDTA, 0.2% triton X100, 0.5 mM Dithiothreitol, protease inhibitor cocktail, pH 7.9) and incubated on ice for 20 min. The tissue homogenates were vortexed and centrifuged (14,000 g; 10 min; 4 °C), and the supernatants were retained for analysis [22].

Determination of Oxidative Stress Biomarkers

Oxidant and antioxidant factors were measured in tissue homogenate. Tissue levels of total antioxidant capacity (TAC) and total oxidant status (TOS) and thiol groups were measured by spectrophotometric methods. Lipid peroxidation levels in the kidney tissue were measured by fluorometric method. Nitric oxide (NO) content in kidney tissue was determined using a commercial kit (ZellBio GmbH (Germany) according to the manufacturer's instructions.

Determination of Gene Expression

Total RNA was extracted using RNX-Plus reagent and cDNA was synthesized using a Prime Script 1st strand cDNA synthesis kit (Takara Bio Inc., Kyoto, Japan). QRT-PCR was performed using SYBR Green (an asymmetrical cyanine dye) premix Ex Taq™ II (TaKaRa Biotechnology, Japan) on a Roche Light Cycler 96 System (Roche Life Science Deutschland GmbH, Sandhofer, Germany). The relative copy number was calculated using the threshold crossing point (Ct) as calculated by $2^{-(\Delta\Delta C_t)}$ formula. Primer sequences were as follows: F: ACTCCTGCAGACTGGAATGG and R: CAAAGCTCAGAGAGCCCATC for KIM; F: CCG GAT TGT GCT TGA GAA CA and R: CTC ACT AGG CAA CTC GCC CA for ELMO-1; F: TCA TTG ACC TCA ACT ACA and R: CAA AGT TGT CAT GGA TGA CC for β -actin.

Histopathological Studies

For histopathological examination, formalin-fixed kidney tissue samples were embedded in paraffin, sectioned at 5 μ m thicknesses and stained with hematoxylin–eosin for light microscopic examination [23].

Statistical Analysis

All data were expressed as mean $\pm$ SD. Data were analyzed using one-way ANOVA followed by post hoc analysis using the Tukey range test (SPSS 16.0 statistical software). The *P* values less than 0.05 were considered to be statistically significant.

Results

General Evaluations

Effects of silymarin on body weight, fasting blood glucose, serum insulin and HOMA-IR after 60 days of treatments are summarized in Table 1. Fasting blood glucose and HOMA-IR in diabetic rats showed a significant increase

($P < 0.001$) compared with healthy controls. Fasting blood glucose and HOMA-IR index reduced significantly in the treated groups (FBS: $P < 0.001$; HOMA-IR: $P < 0.01$, Table 1). In addition, the level of insulin secretion and the body weight in the diabetic control group was significantly decreased ($P < 0.001$). Treatment with different doses of silymarin increased insulin levels ($P < 0.001$) and body weight in rats compared to diabetic controls.

Biochemical Parameters

The levels of urea, creatinine, uric acid and blood urea nitrogen (BUN) in the diabetic group significantly increased compared with the control group ($P < 0.001$, Table 2). The effect of silymarin on urine parameters were shown in Table 3. The levels of urea; uric acid; urine volume; kidney weight and protein levels increased in diabetic rats when compared with healthy control rats ($P < 0.001$). Administration of silymarin at both doses (60 and 120 mg/kg) reduced urea; uric acid; urine volume and kidney weight levels significantly.

Oxidative Stress Biomarker

Effect of silymarin on the kidney oxidative stress factors after 60 days of treatments were shown in Table 4. Oxidative stress factors in the kidney tissue were measured after 60 days of silymarin treatment. Silymarin (120 mg/kg) treatment showed a significant reduction ($P < 0.001$) in the kidney TOS, MDA and NO contents. TAC and thiol group levels in the kidney tissues were significantly increased in the treated groups compared with diabetic group ($P < 0.001$).

Effects of Silymarin on ELMO1 and KIM1 Gene Expression

As shown in Fig. 1 the expression of ELMO and KIM significantly increased in diabetic group compared with healthy control rats after 8 weeks of receiving NIC/STZ ($P < 0.001$). Administration of 60 and 120 mg/kg of silymarin could completely decreased the expression levels of ELMO and KIM in silymarin-treated rats compared with the diabetic group (ELMO1; group 5 vs. group 4 $P < 0.01$; and group 6 vs. group 4, $P < 0.001$, Fig. 1); (KIM1, group 5 vs. group 1, $P < 0.001$; and group 6 vs. group 4, $P < 0.001$, Fig. 1).

Histological Observations

As shown in Fig. 2, in the kidney tissues of control group, the proximal and distal convoluted tubules, renal corpuscles, glomerulus and glomerular capsule had normal

Table 1 Effect of silymarin on rats' body weight, fasting blood glucose, serum insulin and HOMA-IR after 60 days of treatments

Parameter	Group I (n = 6)	Group II (n = 6)	Group III (n = 6)	Group IV (n = 6)	Group V (n = 6)	Group VI (n = 6)
Initial body weight (g)	230.51 ± 8.59	225.16 ± 7.52	228.50 ± 9.94	226.33 ± 7.73	229.66 ± 8.61	228.16 ± 4.91
Final body weight (g)	297.50 ± 18.39	288.00 ± 18.69	289.00 ± 19.76	182.50 ± 16.94a,*	215.6 ± 14.94a,*,b,+	219.83 ± 23.69a,*,b,+
FBS (mg/dl), pretreatment	76.88 ± 7.08	72.33 ± 4.92	79.50 ± 7.73	76.83 ± 6.85	72.33 ± 4.84	77.86 ± 5.07
FBS (mg/dl), the first day of treatment	76.02 ± 6.99	76.00 ± 7.07	74.33 ± 8.45	220.16 ± 6.94a,*	218.83 ± 6.17a,*	216.33 ± 9.51a,*
FBS (mg/dl), the last day of treatment	83.33 ± 10.93	76.50 ± 12.24	79.50 ± 13.80	299.383 ± 27.05a,*	202.67 ± 45.14b,*	182.50 ± 35.82b,*
Insulin (μU/ml)	11.46 ± 0.47	11.96 ± 0.67	11.65 ± 1.64	7.10 ± 1.06a,*	9.60 ± 1.11b,*	9.80 ± 0.96b,*
HOMA-IR	2.35 ± 0.27	2.24 ± 0.26	2.28 ± 0.41	5.79 ± 0.90a,*	4.71 ± 0.64b,+	4.35 ± 0.56b,†

Results were expressed as mean ± SD. Group I: normal control group which received normal saline, group II: normal control group which received silymarin 60 mg/kg, group III: normal control group which received silymarin 120 mg/kg, group IV: diabetic control group which received normal saline, group V: diabetic group which received silymarin 60 mg/kg. Group VI: diabetic group which received silymarin 120 mg/kg

FBS fasting blood sugar

+ $P < 0.05$; † $P < 0.01$; * $P < 0.001$

a Significantly compared to the healthy control group

b Significantly compared to the diabetic control group

Table 2 Effect of silymarin on serum variables values after 60 days of treatments

Parameter	Group I (n = 6)	Group II (n = 6)	Group III (n = 6)	Group IV (n = 6)	Group V (n = 6)	Group VI (n = 6)
Uric acid (mg/dl)	3.01 ± 0.75	2.89 ± 0.58	3.05 ± 0.62	5.19 ± 0.81a,*	4.72 ± 0.42a,†	3.69 ± 0.33b,†
Creatinine (mg/dl)	0.83 ± 0.10	0.93 ± 0.13	0.87 ± 0.16	2.26 ± 0.71a,*	1.53 ± 0.48a,†,b,†	1.27 ± 0.22b,†
Urea (mg/dl)	29.61 ± 5.05	30.50 ± 6.41	32.66 ± 4.80	57.83 ± 9.57a,*	44.72 ± 5.34a,†,b,†	37.72 ± 6.84b,*
BUN (mg/dl)	13.84 ± 2.36	14.25 ± 2.99	15.26 ± 2.24	27.02 ± 4.47a,*	20.89 ± 2.49a,†,b,†	17.62 ± 3.19b,*
Total protein (g/dl)	6.51 ± 1.01	6.75 ± 0.64	6.63 ± 0.34	6.22 ± 0.23	6.18 ± 0.66	6.06 ± 0.41

Results were expressed as mean ± SD. Group I: normal control group which received normal saline, group II: normal control group which received silymarin 60 mg/kg, group III: normal control group which received silymarin 120 mg/kg, group IV: diabetic control group which received normal saline, group V: diabetic group which received silymarin 60 mg/kg. Group VI: diabetic group which received silymarin 120 mg/kg

+ $P < 0.05$; † $P < 0.01$; * $P < 0.001$

a Significant compared to the healthy control group

b Significantly compared to the diabetic control group

architecture. Additionally, the bowman capsule and glomerulus had a normal size. Histopathological examination of the kidneys from untreated diabetic rats showed mild tubular degeneration and necrosis characterized by eosinophilic cytoplasm with pyknotic nuclei, mild tubular cytoplasmic vacuolations, degenerative and necrotic changes in the glomerular epithelium, multifocal mononuclear cell infiltration in the interstitial tissue,

congestion and diffused glomerular and interstitial hemorrhages. Furthermore, cortical tubular and glomerular hypertrophy and an apparent increase in the thickness of bowman capsules were observed.

In the treated diabetic rats, oral administration of different doses of silymarin reduced degenerative and necrotic changes in the tubular and glomerular epithelium and also glomerular and interstitial hemorrhages. Tubular epithelial

Table 3 Effect of silymarin on urine variables values after 60 days of treatments

Parameter	Group I (n = 6)	Group II (n = 6)	Group III (n = 6)	Group IV (n = 6)	Group V (n = 6)	Group VI (n = 6)
Urine volume (ml/day)	9.16 ± 2.48	11.41 ± 2.20	11.33 ± 2.94	30.66 ± 10.03a,*	24.33 ± 5.71a,*	17.16 ± 2.60b,#
Uric acid (mg/day)	0.38 ± 0.12	0.54 ± 0.14	0.48 ± 0.12	5.76 ± 1.02a,*	2.94 ± 0.84a,#,b,*	1.10 ± 0.22 b,*
Creatinine (mg/dl)	52.66 ± 5.16	55.50 ± 8.01	59.33 ± 7.33	27.66 ± 7.20a,*	33.50 ± 6.80a,*	43.16 ± 6.79b,*
Urea (g/day)	0.16 ± 0.05	0.21 ± 0.04	0.29 ± 0.08	1.41 ± 0.59a,*	0.90 ± 0.21a,#,b,+	0.46 ± 0.04b,*
Kidney weight (gr)	0.81 ± 0.08	0.80 ± 0.04	0.86 ± 0.03	1.29 ± 0.10a,*	1.07 ± 0.09a,*b,#	0.95 ± 0.11b,*
Albumin secretion (mg/day)	3.28 ± 0.41	2.72 ± 0.44	2.54 ± 0.50	8.17 ± 0.28a,*	6.01 ± 0.32a,*b,*	4.02 ± 0.31b,*

Results were expressed as mean ± SD. Group I: normal control group which received normal saline, group II: normal control group which received silymarin 60 mg/kg, group III: normal control group which received silymarin 120 mg/kg, group IV: diabetic control group which received normal saline, group V: diabetic group which received silymarin 60 mg/kg. Group VI: diabetic group which received silymarin 120 mg/kg

GFR glomerular filtration rate

a Significant compared to the healthy control group

b Significantly compared to the diabetic control group

+*P* < 0.05; #*P* < 0.01; **P* < 0.001

Table 4 Effect of silymarin on the kidney oxidative stress factors after 60 days of treatments

Parameter	Group I (n = 6)	Group II (n = 6)	Group III (n = 6)	Group IV (n = 6)	Group V (n = 6)	Group VI (n = 6)
NO (μmol g ⁻¹)	1.62 ± 0.26	1.89 ± 0.15	1.64 ± 0.27	4.84 ± 0.40a,*	3.32 ± 0.42a,*b,*	2.39 ± 0.35b,*
MDA (pmol/mg protein)	238.33 ± 36.87	234.17 ± 29.73	229.00 ± 24.29	466.67 ± 58.25a,*	334.83 ± 28.24b,*	292.33 ± 15.68b,*
TTM (nmol/mg protein)	139.58 ± 8.89	144.08 ± 1.42	146.32 ± 1.79	110.33 ± 1.63a,*	118.08 ± 6.24a,*	136.58 ± 3.62b,*
TOS (nmol/mg protein)	11.09 ± 0.76	12.20 ± 0.80	12.30 ± 1.35	19.18 ± 1.69a,*	13.80 ± 0.67b,*	8.03 ± 1.54b,*
TAC (nmol/mg protein)	133.17 ± 0.98	134.00 ± 2.36	142.42 ± 3.13	96.25 ± 4.64a,*	100.42 ± 3.47a,*	137.49 ± 3.17b,*
OSI (TOS/TAC)	0.07 ± 0.008	0.085 ± 0.005	0.081 ± 0.007	0.19 ± 0.24a,*	0.13 ± 0.008a,*b,*	0.053 ± 0.012b,*

Results were expressed as mean ± SD. Group I: normal control group which received normal saline, group II: normal control group which received silymarin 60 mg/kg, group III: normal control group which received silymarin 120 mg/kg, group IV: diabetic control group which received normal saline, group V: diabetic group which received silymarin 60 mg/kg. Group VI: diabetic group which received silymarin 120 mg/kg

MDA malondialdehyde, *TTM* total thiol molecule, *TOS* total oxidant status, *TAC* total antioxidant capacity, *OSI* oxidative stress index

a Significant proportion of the healthy control group

b Significantly compared to the diabetic control group

+*P* < 0.05; #*P* < 0.01; **P* < 0.001

hypertrophy and mononuclear cell infiltration were significantly lower in the treated diabetic rats compared to the diabetic group. There was no evidence of tubular vacuolations and the thickness of bowman capsules was normal.

Kidney sections of normal rats treated with different doses of silymarin showed no pathological alterations and were comparable to those of control rats.

Discussion

T2DM or non-insulin dependent diabetes is an important metabolic disease in the world. Due to the multifactorial nature of the disease, various factors contribute to it. Due to decreased insulin sensitivity in type 2 diabetes, altered glucose homeostasis is caused, which ultimately leads to hyperglycemia [24]. In this study we investigated useful effects of silymarin on kidney of type 2 diabetic rats.

Fig. 1 Effect of silymarin (60 and 120 mg/kg) on **a** ELMO1 and **b** KIM1 mRNA levels. Results were expressed as mean $\pm$ SD. Group I: normal control group which received normal saline, group II: normal control group which received silymarin 60 mg/kg, group III: normal control group which received silymarin 120 mg/kg, group IV:

diabetic control group which received normal saline, group V: diabetic group which received silymarin 60 mg/kg. Group VI: diabetic group which received silymarin 120 mg/kg. **a** Significant proportion of the healthy control group. **b** Significantly compared to the diabetic control group, $^{\#}P < 0.01$ and $^{*}P < 0.001$

Our results showed decreased levels of glucose, urea, creatinine and uric acid in serum and urine parameters in treated diabetic groups in compared with untreated groups. Other works about silymarin effects on diabetes condition have performed. Extract of *S. marianum* can undermine diabetic kidney damage by lowering proteinuria, for its antioxidant function. This property was also reported with silymarin, as a dominant form of flavonolignan in extract of milk thistle. Treatment with various doses and longer periods of silymarin and evaluation of kidney function is suggested in future [25]. It was shown that silymarin extract could safely be used together with metformin to increase the antioxidant potency and better reno-protection. Therefore, combination of silymarin with other chemical drugs may have additive kidney protective property beyond controlling the blood sugar of drugs such as metformin, to prevent or slowing the progression of diabetic nephropathy [26].

Our finding in oxidative stress experiments showed important and significant antioxidant effects and oxidative reducing properties of silymarin. In line with our results, the study of Vessal and colleagues in 2010 showed that silymarin may prevent the development of diabetic nephropathy (DN) in diabetic rats. Milk thistle extract may decrease nephropathy in rats by reduction of oxidative stress and increasing antioxidant enzyme levels [25]. Ahmadi et al. demonstrated that silymarin in patients with hemodialysis, significantly reduced serum MDA concentrations, increased hematocrit index and glutathione peroxidase levels. Silymarin also diminish kidney injury and repairs the activity of antioxidant enzymes in diabetic rats. In STZ-induced diabetic rats, milk thistle extract can decrease and suppress diabetic nephropathy by increasing

catalase and glutathione peroxidase activity and decreasing lipid peroxidation in renal tissue [27].

We demonstrated an increased expression of ELMO1 in diabetic animals. There are other studies in supporting our work. Studies have shown that expression of ELMO1 is increased in patients of renal glomerulus damage, resulting in increasing of extracellular matrix proteins and loss of cell adhesive cellular [28]. ELMO-1 plays an important role in the development of diabetic nephropathy. Also, with an increased mRNA expression of ELMO-1, the production of ROS increases and is possibly due to increased oxidative stress causing kidney impairment [29]. Other studies have shown that ELMO1 to be associated with diabetic nephropathy in Japanese patients with type 2 diabetes [28]. Our result for the first time showed beneficial effects of silymarin on ELMO1 expression.

Our finding showed increased levels of mRNA transcript of KIM1 in the kidney tissue of diabetic rats and administration of both doses of silymarin reduced KIM1 expression. In a study by Wang et al. the protective effect of Vorinostat against renal damage induced by calcium oxalate in mice was investigated. The study found that KIM1 is known as a sensitive and specific marker for renal injury. The results showed that KIM1 urinary levels in mice that consumed saline and dimethyl sulfoxide were higher than those in other groups, while serum creatinine levels remained unchanged. These results indicate that increasing KIM1 level in relation to serum creatinine may have a positive role in detecting renal damage in the early stages [30].

In summary based on our finding silymarin showed kidney-protective effects via reduction of oxidative stress and down regulation of ELMO1 and KIM1 in the kidney

Fig. 2 Histological sections of kidney from the untreated diabetic rats and diabetic rats treated with silymarin.

a Diabetic rat. Tubular degeneration and necrosis (arrows) (**h, e**; bar = 100 μ m). **b** Diabetic rat. Cytoplasmic vacuolations of the epithelial cells in the cortical renal tubules (arrows) (**h, e**; bar = 25 μ m). **c** Diabetic rat. Glomerular hypertrophy (arrowheads) (**h, e**; bar = 100 μ m). **d** Diabetic rat. Cortical tubular hypertrophy (**h, e**; bar = 25 μ m). **e** Infiltration of mononuclear cell in the interstitial tissue (**h, e**; bar = 100 μ m). **f** Glomerular degeneration with enlargement of bowman capsules (stars) (**h, e**; bar = 25 μ m). **g** Treated diabetic rat. Mild tubular and glomerular hypertrophy with minimal degenerative changes (**h, e**; bar = 100 μ m). **h** Mild hypertrophy of tubules without cytoplasmic vacuoles (**h, e**; bar = 25 μ m)

tissues of type 2 diabetic rats. More studies required to reveal exact molecular mechanism of silymarin.

Acknowledgements This study was financially supported by the Hamadan University of Medical Sciences (No: 9510075671). The results presented in this article were F. Goli. M.Sc. thesis.

Compliance with ethical standards

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

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