ORIGINAL ARTICLE

Silibinin, a bioactive flavanone, prevents the progression of early diabetic nephropathy in experimental type-2 diabetic rats

Dilpesh P. Jain, Rahul S. Somani[1]
Departments of Research and [1]Pharmaceutical Sciences, Suresh Gyan Vihar University, Jaipur, Rajasthan, India

Aim: Silibinin, also known as silybin, is the major active constituent of silymarin reported myriad pharmacological activities. The present study investigates the protective effect of silibinin in diabetic nephropathy (DN) in experimentally induced type-2 diabetic rats. **Materials and Methods:** Oral glucose tolerance test was performed in Sprague Dawley rats, fed with high-fat diet for 2 weeks. Rats failed to compensate glucose load were rendered diabetic with streptozotocin (35 mg/kg; i.p) and left untreated for 4 weeks. Thereafter, diabetic rats were orally treated with silibinin (40 or 80 mg/kg) for 4 weeks. **Results:** A significant hyperglycaemia and hyperlipidaemia were observed in diabetic rats as compared to control rats, and these changes were significantly restored in the silibinin treated diabetic rats. Further a long standing hyperglycaemia resulted in altered antioxidant system and induced DN, characterised by increased serum and urinary creatinine ($P < 0.001$), urea nitrogen ($P < 0.001$), creatinine clearance ($P < 0.001$) and urinary albumin excretion rate ($P < 0.001$) as well as decreased albumin ($P < 0.01$) and total protein ($P < 0.001$). Restoration of kidney functions and antioxidant system were observed in the diabetic rats treated with silibinin. Moreover, histopathological alterations in diabetic rats were restored towards near normal architecture with silibinin treatment. **Conclusion:** The present study indicated that 4 weeks of silibinin treatment may prevent the progression of early DN.

Key words: Creatinine, diabetes, nephropathy, silibinin, streptozotocin

INTRODUCTION

Diabetic nephropathy (DN) is a microvascular complication of chronic diabetes pervading in the world to evolve as a global pandemic. Recent estimate state in 2011 there were 366 million people had diabetes worldwide, and it is expected to rise 552 million by 2030.[1] DN characterised by pathophysiologic perturbations of glomerular filtration rate (GFR), that is, hyper-filtration, and albumin excretion, that is, micro-albuminuria, followed by proteinuria, nephrosis, azotaemia and finally end-stage renal disease.[2] Accumulating evidences indicate DN results due to a complex interplay between haemodynamic and metabolic factors.[3,4] Chronic hyperglycaemia increases the activity of endothelin as well as renin angiotensin aldosterone system. Increased systemic and intraglomerular pressure is responsible for the overproduction of reactive oxygen species, release of cytokines (tumour necrosis factor-α, Interleukin [IL-6], IL-18) and growth factors (transforming growth factor beta, vascular endothelial growth factor) that leads to mesangial expansion and glomerulosclerosis.[4,5] Moreover, glucose mediated activation of intracellular second messengers such as protein kinase C and nuclear transcription factor Nuclear factor-kappaB reported to cause the tubular inflammation, cell proliferation, and glomerular hypertrophy and thus implicated in the development of DN.[5-7]

Current scenario suggests persistent rise in nephropathy associated with diabetes and till date no satisfactory option available except fewer agents like angiotensin converting enzyme inhibitors and angiotensin receptor blockers.[8] However, traditional medicines, mainly from plant sources are found to be effective in the management of diabetes.[9,10] Recently, *in vitro* and *in vivo* preclinical studies reported positive effects of flavonoids in halting the progression and/or development of diabetic kidney disease.[8] Silibinin, a flavanone is the major and most active component of silymarin.[11] It has been reported as an anti-diabetic.[12,13] Various preclinical reports suggested the myriad pharmacological activities of silibinin. It has been reported as an antioxidant and hepatoprotective in non-alcoholic steatohepatitis.[14] Silibinin markedly improved endothelial dysfunction in db/db mice by

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.155060

Address for correspondence: Mr. Dilpesh Jain, Department of Research, Suresh Gyan Vihar University, Mahal Jagtpura, Jaipur - 302 001, Rajasthan, India. E-mail: dilpeshjain@yahoo.com
Received: 30-01-2015; **Accepted:** 18-03-2015

reducing circulating and vascular ADMA levels.[15] Recently, Marrazzo *et al*., reported its neuroprotective effect due to DNA protection and antioxidant activity in diabetic mice.[16] However, it largely remains unclear whether the treatments of silibinin ameliorate DN associated with type-2 diabetes. We therefore, investigated the protective effect of silibinin on early DN in experimentally induced type-2 diabetic rats.

MATERIALS AND METHODS

Drugs and Chemicals

Silibinin, malondialdehyde (MDA), tetrabutyl ammonium and superoxide dismutase (SOD) (Sigma-Aldrich, St. Louis), streptozotocin (STZ) (Enzo Life Sciences, UK), captopril (Wockhardt Ltd, India), commercial diagnostic kits (Biolab, India) and all other reagents and chemical were of analytical grade and purchased from local suppliers of Pune, India.

Experimental Animals

Sprague Dawley (SD) rats (150–200 g) were procured from National Institute of Biosciences, Pune. Rats were placed separately in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23°C ± 2°C with relative humidity 55% ±10% in a 12 h light and 12 h dark cycle throughout the experiment. Animals had free access to water and standard laboratory feed (Nutrivet Lab, India). All the experimental procedures and protocols used in this study were reviewed and approved (IAEC/2011-12/33) by the Institutional Animal Ethics Committee, constituted under committee for the purpose of control and supervision of experiments on animals by Ministry of Environment and Forests, Government of India, New Delhi, India. Ethical guidelines were strictly followed during all the experimental procedures.

Induction of Experimental Diabetes

After 1-week of acclimatisation, type-2 diabetes was induced in SD rats with a little modification.[17,18] Animal were fed with a combination of high-fat emulsion (HFE) and high-fat diet (HFD) which was manipulated from normal pellet diet for 2 weeks. Whereas the age matched, control animals were received normal pellet diet only. HFE and HFD were prepared in such way that total calories obtained from fat were of 60%. After 2 weeks of administration of HFE and HFD, oral glucose tolerance test was performed to check insulin resistance. The animal failed to compensate glucose load were then rendered diabetic with a single intraperitoneal injection of STZ (35 mg/kg, body weight). STZ was freshly prepared in cold citrate buffer (pH 4.4, 0.1 M). Control rats were injected with cold citrate buffer (pH 4.4, 0.1 M) only. 48 h after STZ injection, diabetes was confirmed by measuring fasting blood glucose levels using a glucometer (Contour TS, Bayer Healthcare, India). Rats with blood glucose level ≥ 200 mg/dl were considered as diabetic and used for further study. During the course of the experiment, the normal and diabetic rats were fed with normal diet and HFD respectively.

Experimental Design

A 4 weeks after diabetes confirmation, diabetic and age matched normal rats were randomly divided into five groups (n=6): Control, diabetic, diabetic + silibinin (40 mg/kg), diabetic + silibinin (80 mg/kg) and diabetic + captopril (100 mg/kg). Silibinin and captopril were suspended/dissolved in carboxymethylcellulose (CMC) (0.01 g/ml) and was daily administered by oral gavage for 4 weeks. Control and diabetic rats were received CMC alone.

Sample Collection

After the last dose of treatment (28th day), rat was individually placed in the metabolic cage, and urine was collected. Blood was withdrawn from the retro-orbital plexus using a micro-capillary technique under light anaesthesia, centrifuged at 3000 rpm for 15 min and serum was separated.

Oral Glucose Tolerance Test

An overnight fasted rats were received a glucose load by oral gavage at a dose of 2 g/kg body weight. Blood was withdrawn from tail vain by pricking tail tip and blood glucose was estimated using glucometer (Contour TS, Bayer Healthcare, India) at 0, 30, 60, 90 and 120 min after glucose load.

Body and Kidney Weight Change

The body weight of all animals after the experiment was taken, and their difference was expressed as body weight change. After sacrificing the animal one of the kidneys was rinsed in chilled saline, decapsulated blotted on filter paper and quickly weighed. For standardisation, total kidney weight was normalised as kidney/body-weight ratio.

Relative kidney weight (%) = [absolute kidney weight/body weight at sacrifice] ×100.

Biochemical Estimations in Serum and Urine

Fasting glycaemia was measured before and at the end of treatment (28 days) using glucometer. Serum levels of total cholesterol, triglycerides, urea nitrogen, creatinine, albumin and total proteins were estimated spectrophotometrically using commercial diagnostic kits. Urine components were determined as 24 h total urine volume, creatinine, and albumin, whereas creatinine clearance (Ccr) and urinary albumin excretion rate (UAER).

Evaluation of Oxidative Stress

At the end of experiment rats were sacrificed by decapitation and kidneys were excised, washed

immediately with ice cold physiological saline (0.9% Nacl) and weighed. Right, kidney was taken for histopathological examination while left kidney was homogenised in chilled 50 mM phosphate buffer saline (pH 7.4). The homogenates were centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was used to determine the concentration of MDA[19] and reduced glutathione (GSH)[20] and SOD[21] and Catalase (CAT)[22] activity. Protein concentrations of homogenates were determined according to Lowry *et al.*[23]

Histopathological Examination

Renal tissues were fixed in 10% formalin, processed routinely, and embedded in paraffin. 5-µm thick sections were prepared, stained with periodic acid-Schiff (PAS) and observed under the light microscope.

Statistical Analysis

The results were expressed as mean ± standard error of the mean. The data were analysed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests and two-way ANOVA, followed by Bonferroni posttest. $P \leq 0.05$ was considered statistically significant.

RESULTS

Oral Glucose Tolerance Test

Oral glucose load produced significant elevation in the blood glucose levels in HFE and HFD fed rats at various time intervals 30 (14.86%), 60 (28.83%), 120 (41.14%) and 180 (61.92%) min compared to control rats. However, control rats showed complete recovery at 120 min after glucose load [Figure 1].

Body Weight, Kidney Weight and Relative Kidney Weight

A significant decrease in body weight and increased kidney weight, and relative kidney weight were noticed in diabetic rats as compared to control rats ($P < 0.001$). Silibinin treatment (40 or 80 mg/kg) in diabetic rats significantly restored the body weight, kidney weight and relative kidney weight [Table 1].

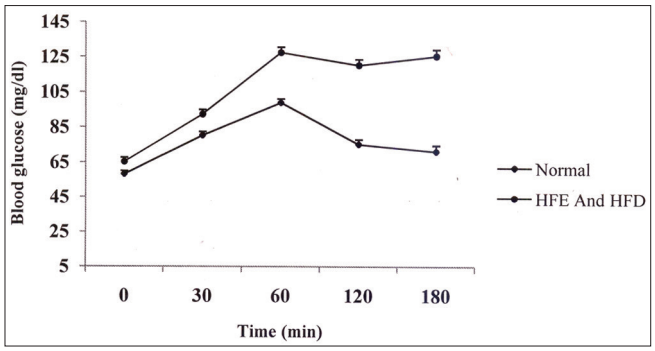

Figure 1: Effect of silibinin on oral glucose tolerance curves. **$P<0.01$ compared to control rats (Two-way ANOVA followed by Bonferroni posttest). HFD: High-fat diet and HFE: High-fat emulsion

Biochemical Estimations in Serum and Urine

As shown in Figure 2, diabetic rats exhibit significant rise in blood glucose level ($P < 0.001$) than control rats, however silibinin treated diabetic rats (40 or 80 mg/kg) significantly decreased the elevated blood glucose levels ($P < 0.001$). Further, serum levels of total cholesterol, triglycerides, blood urea nitrogen (BUN) and creatinine and urinary creatinine were found to be higher in diabetic rats when compared with control rats ($P < 0.001$). Diabetic rats treated with silibinin (40 or 80 mg/kg) significantly reduced the elevated total cholesterol and triglycerides levels ($P < 0.001$) whereas; BUN and serum and urinary creatinine were reduced dose dependently. Moreover, decreased serum albumin and total protein levels in diabetic rats were significantly restored with silibinin treatment at a dose level of 80 mg/kg ($P < 0.05$ and $P < 0.01$, respectively) [Table 2].

24 h total urine volume, Ccr and UAER were significantly increased in diabetic rats than control rats. Diabetic rats treated with silibinin (40 or 80 mg/kg) significantly reduced urine volume ($P < 0.001$), Ccr ($P < 0.01$ and $P < 0.001$; respectively) and UAER ($P < 0.001$) [Figures 3-5].

Oxidative Stress

As shown in Table 3, increased MDA concentration and decreased SOD and CAT activities as well as GSH concentration was found in diabetic rats ($P < 0.001$) when compared with control rats. 4 weeks of silibinin

Table 1: Effect of silibinin on body weight and kidney weight change

Group	Body weight (g)	Kidney weight (g)	Relative kidney weight (%)
Control	228±2.00	0.95±0.06	0.41±0.019
Diabeti (D)	168±2.20[a]	1.71±0.10[a]	1.01±0.054[a]
D + silibinin (40)	205±4.46[#]	1.30±0.07[@]	0.63±0.040[#]
D + silibinin (80)	215±4.54[#]	0.99±0.03[#]	0.46±0.022[#]
D + captopril (100)	188±6.10[@]	1.20±0.07*	0.63±0.023[#]

[a]$P<0.001$ versus control rats, [@]$P<0.05$, *$P<0.01$, [#]$P<0.001$ versus diabetic rats

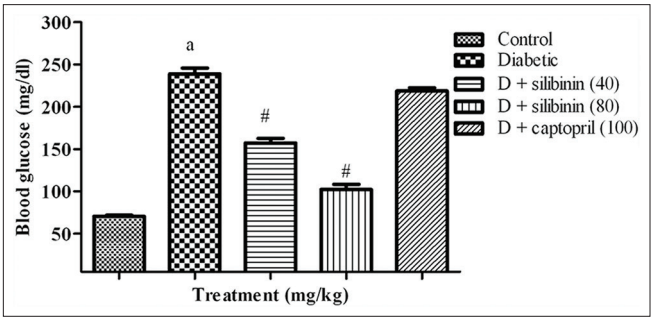

Figure 2: Effect of silibinin on fasting blood glucose levels [a]$P<0.001$ versus control rats, [#]$P<0.001$ versus diabetic rats

Table 2: Effect of silibinin on biochemical estimations

Treatment (mg/kg)	Control	Diabetic (D)	D + silibinin (40)	D + silibinin (80)	D + captopril (100)
TC (mg/dl)	62.0±2.9	137±5.2[a]	103±2.8[#]	71±3.40[#]	139±4.00
Triglyceride (mg/dl)	45±2.7	107±4.7[a]	77±3.4[#]	55±2.2[#]	120±5.3
Urea nitrogen (mg/dl)	28±1.30	51±1.10[a]	43±3.00[@]	32±1.1[#]	40±2.1*
Albumin (g/dl)	3.9±0.15	2.3±0.03[b]	3.1±0.38	3.7±0.20[@]	3.6±0.29[@]
Total protein (g/dl)	8.5±0.22	5.7±0.43[a]	6.0±0.47	7.6±0.18*	7.0±0.13
Serum creatinine (mg/dl)	0.77±0.02	1.40±0.09[a]	1.10±0.04*	0.92±0.04[#]	1.00±0.02[#]
Urinary creatinine (mg/dl)	41.00±3.50	81.00±3.60[a]	64.06±4.48[@]	47.00±1.68[#]	74.81±3.99[#]

[a]P<0.001 and [b]P<0.01 versus control rats, [@]P<0.05, *P<0.01, [#]P<0.001 versus diabetic rats. TC – Total cholesterol

Table 3: Effect of silibinin on renal oxidative stress

Group	MDA (nmol/mg)	SOD (U/mg)	GSH (ng/mg)	CAT (U/mg)
Control	3.82±0.42	22.0±1.2	34.86±1.63	43.86±2.63
Diabetic	12.00±1.00[a]	9.4±0.61[a]	14.97±1.08[a]	31.97±1.58[a]
D + silibinin (40)	8.20±0.84*	12.03±0.92	25.67±1.97[#]	35.67±1.97[#]
D + silibinin (80)	5.80±0.71[#]	18.25±0.83*	30.09±1.15[#]	38.09±1.55[#]
D + captopril (100)	6.20±0.75[#]	16.87±1.40[@]	27.21±1.72[#]	37.21±1.22[#]

[a]P<0.001 versus control rats, [@]P<0.05, *P<0.01, [#]P<0.001 versus diabetic rats. MDA – Malondialdehyde; SOD – Superoxide dismutase; GSH – Glutathione; CAT – Catalase

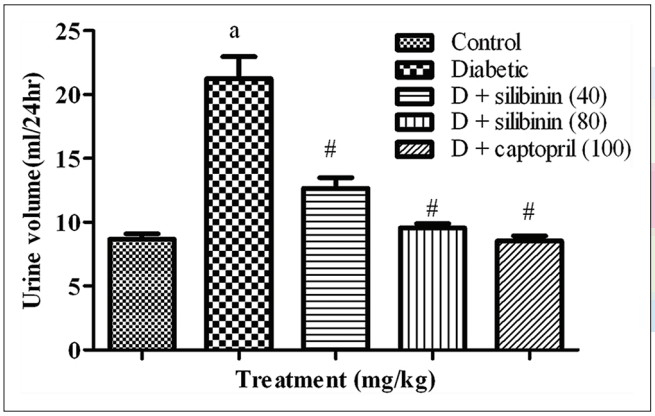

Figure 3: Effect of silibinin on 24 h total urine volume [a]P<0.001 versus control rats, [#]P<0.001 versus diabetic rats

treatment (40 or 80 mg/kg) in diabetic rats significantly decreased the concentration of MDA (P < 0.01 and P < 0.001; respectively) and increased activities of SOD (ns and P < 0.01; respectively) and CAT (P < 0.001 and P < 0.001; respectively) and GSH concentration (P < 0.001 and P < 0.001; respectively). Further, captopril treatment in diabetic rats significantly restored the oxidative stress.

Histopathological Examinations

In PAS stained images of glomeruli, after 10 weeks of study, diabetic rats showed a significant enlargement of the glomeruli, thickening of glomerular basement membranes without nodules and tubular dilatation, while normal control animals were revealed no abnormalities. The treatment of diabetic rats with silibinin for 4 weeks dose-dependently attenuated these progressions compared with diabetic control rats [Figure 6].

DISCUSSION

Flavonoids are known for their therapeutic potential and recently some of them have got the attention in the treatment of diabetes and long standing diabetic complications. Therefore, the present study was designed to investigate the protective effect of silibinin in the development of DN in type-2 diabetic rat models.

Earlier studies have reported rats fed with HFD and administered with low dose of STZ induced type-2 diabetes and DN within 4–6 weeks.[10] Estimation of fasting blood glucose levels is a central basal parameter for monitoring diabetes.[24] Moreover, earlier reports indicate importance of control over the hyperglycaemia by insulin treatment to prevent renal hypertrophy and subsequent increase in urinary protein excretion.[25] STZ induced diabetic rats showed a reduction in body weight due to increased muscle wasting and loss of tissue proteins which is in agreement with the previous reports.[26,27] Silibinin treatments for 4 weeks significantly restored the body weight. DN is characterised by increased accumulation of extracellular matrix proteins and mesangial expansion, responsible for hypertrophy of kidney.[28] In the present study, increased kidney weight and relative kidney weight in diabetic rats were significantly decreased with the 4 weeks of silibinin treatment.

Insulin has an inhibitory action on 3-hydroxy-3-methylglutaryl-coenzyme A reductase, a rate-limiting enzyme responsible for the metabolism of cholesterol-rich low-density lipoprotein particles indicates, correlation of insulin deficiency or insulin resistance with dislipidemia. Moreover, it was reported that the elevated levels of lipids in diabetic mellitus increase the risk of DN.[29,30] Furthermore, it was hypothesised that increased lipotoxicity, that is, increased intracellular concentration of fatty acids induces glomerular and tubular dysfunction in diabetes.[31] Marcovecchio *et al.*, has demonstrated the relation between abnormal lipid profile and risk of development of DN.[32] In the present study elevated levels of serum triglyceride and total cholesterol in diabetic rats were significantly reduced with the 4 weeks treatment of silibinin, which may be contributed to its beneficial effects in DN.

In general creatinine level is considered to assess kidney function.[33] A significant increase in the levels of serum creatinine and BUN in diabetic rats were found to be associated with interstitial atrophy, epithelial necrosis as well as atrophic changes in glomeruli.[34-36] In the present study diabetic rats after 4 weeks, treatment of silibinin showed recovery towards normal levels. While in the line with the previous reports captopril treatment effectively reduced these levels.[37]

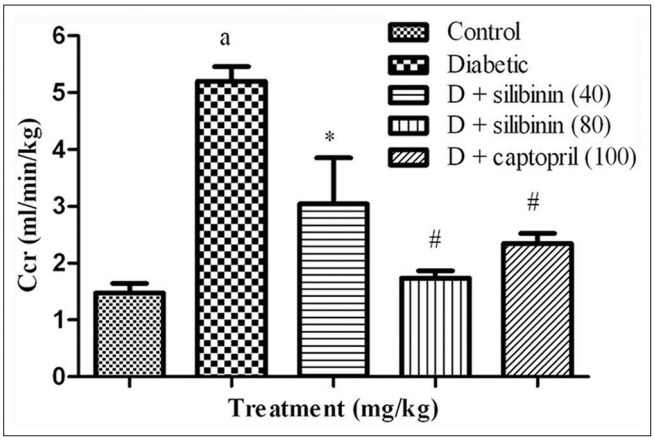

Figure 4: Effect of silibinin on creatinine clearance $^{a}P<0.001$ versus control rats, $^{*}P<0.01$, $^{\#}P<0.001$ versus diabetic rats

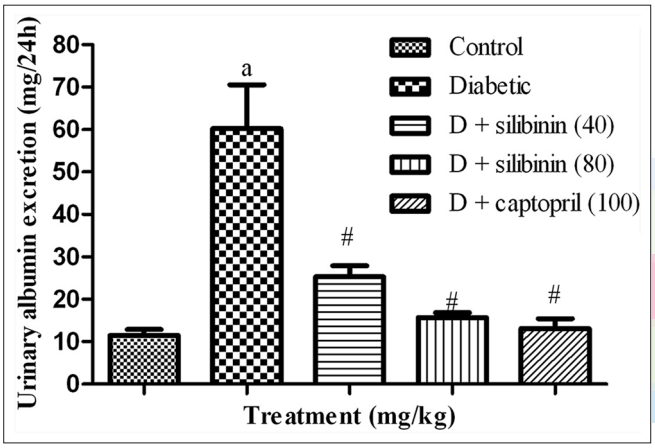

Figure 5: Effect of silibinin on urinary albumin excretion rate $^{a}P<0.001$ versus control rats, $^{*}P<0.01$, $^{\#}P<0.001$ versus diabetic rats

Microalbuminuria and UAER are the major predictor of nephropathy in diabetic patients. It is an indicator of pathophysiological alterations in kidney such as lesions in glomerular basement membrane. After long-standing diabetes, diabetic rats exhibit an increased urinary excretion of albumin indicating progression of DN. 4-weeks treatment of silibinin significantly reduced the microalbuminuria and UAER thus exerted ameliorative effect on diabetic kidney. Further captopril treatment in diabetic rats resulted significant reduction in urinary albumin excretion which is consistent with earlier reports.

Overproduction of prostaglandin E2 was found to be associated with increased GFR in early stages of DN.[38] In addition, a study by Kim *et al*., reported, increased GFR after chronic hyperglycaemia in diabetic rat due to oxidative stress.[39] Long standing diabetes, the result significant increase in urine volume and Ccr in diabetic rats indicate early stage of DN.[40] However, 4 weeks of silibinin and captopril treatment showed a reduction in urine volume and Ccr and recovery towards normal rats.

Chronic hyperglycaemia plays an important role in the production of oxidative stress and thus development of various diabetic complications including DN.[39] Increased level of MDA is an index of endogenous lipid peroxidation,

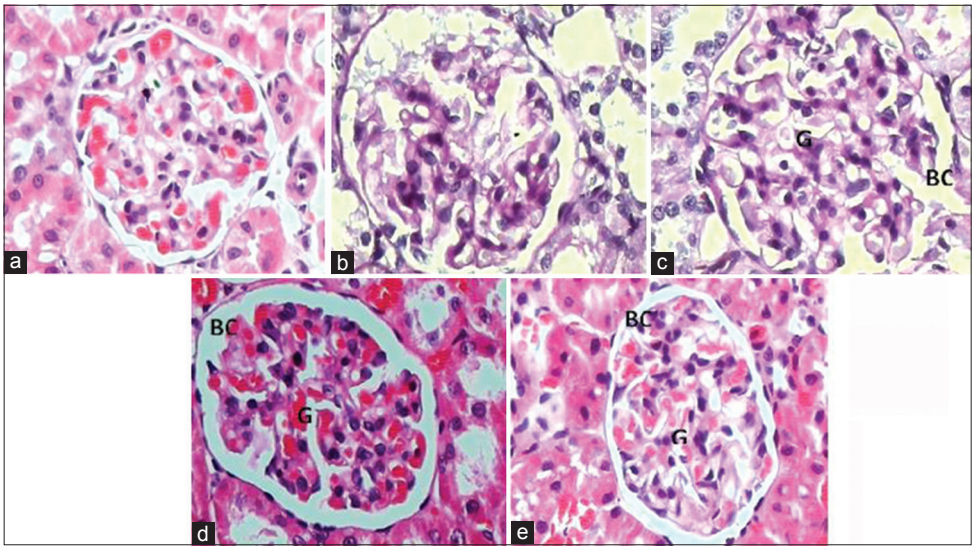

Figure 6: Histological section of kidneys stained with PAS: ×400 (a) normal control (NC) showing no abnormalities, (b) diabetic control (DC) showing an enlargement of the glomeruli, thickening of glomerular basement membranes (GBMs) without nodules and tubular dilatation (c) DC + silibinin (40), (d) DC + silibinin (80) and (e) DC + captopril (100) showing dose-dependent features of healing towards normal basement membrane and mesangial expansion. BC: Bowman's capsule; G: Glomerulus; (mesangial expansion and GBM thickening)

induction of diabetes significantly increased MDA levels, reflecting oxidative stress. Various antioxidants found effective in the treatment and/or prevention of diabetic complications including DN.[40-42] This suggests the key role of oxidative free radicals and thus oxidative stress in the pathogenesis of DN. In harmony, we have observed significant increase in oxidative markers such as MDA and decrease in GSH, SOD, and CAT in kidney tissues. Treatment of silibinin for 4 weeks restored the oxidative stress. The reversal of the oxidative damage due to silibinin indicates that it has possible antioxidant properties, which plays a crucial role in the defence against free oxidative radicals. Histopathological findings revealed that long standing diabetes caused marked alterations in the normal renal architecture. GBM thickening and marked mesangial expansion were observed in diabetic control rats. DN is known to be associated with increased synthesis and/or accumulation of ECM due to decreased degradation of matrix proteins. Increased oxidative stress, overproduction of AGE, stimulation of renin angiotensin system and expression of growth factors and cytokines in kidney are all responsible for mesangial expansion and ECM accumulation.[43] We have observed increased ECM accumulation along with foci of mesangial hypercellularity and capillary basement membrane thickening in kidney of diabetic control rats. Treatment with silibinin substantially decreased these renal changes dose-dependently.

CONCLUSION

In conclusion, the animals fed with HFD for 2 weeks significantly produced the glucose intolerance and persistent hyperglycaemia after STZ injection. Further the persistent hyperglycaemia significantly declines the kidney functions, increases oxidative stress and increased extracellular matrices (ECM) accumulation and thickening of glomerular basement membrane. Silibinin treatment significantly restored the blood glucose, kidney functions, oxidative stress and renal histopathology. All these findings suggest that silibinin has a beneficial role in slowing the progression of early DN in type-2 diabetic rats, which may be due to its multivariate actions such as antihyperglycaemic, antioxidant and antihyperlipidemic.

ACKNOWLEDGEMENT

The authors would like acknowledge Prof. M. N Nawale, President, Sinhgad Technical Education Society and Dr. K N Gujar, Principal, Sinhgad College of Pharmacy, Vadgaon (BK), Pune, India, for providing necessary facilities to carry out the present study.

REFERENCES

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94:311-21.
2. Friedman EA. Evolving pandemic diabetic nephropathy. Rambam Maimonides Med J 2010;1:e0005.
3. Cooper ME. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia 2001;44:1957-72.
4. Zelmanovitz T, Gerchman F, Balthazar AP, Thomazelli FC, Matos JD, Canani LH. Diabetic nephropathy. Diabetol Metab Syndr 2009;1:10.
5. Balakumar P, Arora MK, Ganti SS, Reddy J, Singh M. Recent advances in pharmacotherapy for diabetic nephropathy: Current perspectives and future directions. Pharmacol Res 2009;60:24-32.
6. Barnes PJ, Karin M. Nuclear factor-kappaB: A pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997;336:1066-71.
7. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL. Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. Diabetes 1994;43:1122-9.
8. Lai PB, Zhang L, Yang LY. Quercetin ameliorates diabetic nephropathy by reducing the expressions of transforming growth factor-ß1 and connective tissue growth factor in streptozotocin-induced diabetic rats. Ren Fail 2012;34:83-7.
9. Makni M, Sefi M, Fetoui H, Garoui el M, Gargouri NK, Boudawara T, *et al.* Flax and Pumpkin seeds mixture ameliorates diabetic nephropathy in rats. Food Chem Toxicol 2010;48:2407-12.
10. Somania R, Singhai AK, Shivgunde P, Jain D. Asparagus racemosus Willd (Liliaceae) ameliorates early diabetic nephropathy in STZ induced diabetic rats. Indian J Exp Biol 2012;50:469-75.
11. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs 2001;61:2035-63.
12. Soto C, Recoba R, Barrón H, Alvarez C, Favari L. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comp Biochem Physiol C Toxicol Pharmacol 2003;136:205-12.
13. Soto C, Pérez J, García V, Uría E, Vadillo M, Raya L. Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus. Phytomedicine 2010;17:1090-4.
14. Haddad Y, Vallerand D, Brault A, Haddad PS. Antioxidant and hepatoprotective effects of silibinin in a rat model of nonalcoholic steatohepatitis. Evid Based Complement Alternat Med 2011;2011:nep164.
15. Li Volti G, Salomone S, Sorrenti V, Mangiameli A, Urso V, Siarkos I, *et al.* Effect of silibinin on endothelial dysfunction and ADMA levels in obese diabetic mice. Cardiovasc Diabetol 2011;10:62.
16. Marrazzo G, Bosco P, La Delia F, Scapagnini G, Di Giacomo C, Malaguarnera M, *et al.* Neuroprotective effect of silibinin in diabetic mice. Neurosci Lett 2011;504:252-6.
17. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005;52:313-20.
18. Xu D, Xu M, Lin L, Rao S, Wang J, Davey AK. The effect of isosteviol on hyperglycemia and dyslipidemia induced by lipotoxicity in rats fed with high-fat emulsion. Life Sci 2012;90:30-8.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
20. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
21. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500.
22. Luck H. Methods of Enzymatic Analysis. Vol. III. New York: Academic Press; 1971. p. 885.

23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
24. Maiti R, Das UK, Ghosh D. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. Biol Pharm Bull 2005;28:1172-6.
25. Rasch R, Mogensen CE. Urinary excretion of albumin and total protein in normal and streptozotocin diabetic rats. Acta Endocrinol (Copenh) 1980;95:376-81.
26. Bhutada P, Mundhada Y, Bansod K, Bhutada C, Tawari S, Dixit P, *et al.* Ameliorative effect of quercetin on memory dysfunction in streptozotocin-induced diabetic rats. Neurobiol Learn Mem 2010;94:293-302.
27. Hasanein P, Shahidi S. Effects of combined treatment with vitamins C and E on passive avoidance learning and memory in diabetic rats. Neurobiol Learn Mem 2010;93:472-8.
28. Kanwar YS, Wada J, Sun L, Xie P, Wallner EI, Chen S, *et al.* Diabetic nephropathy: Mechanisms of renal disease progression. Exp Biol Med (Maywood) 2008;233:4-11.
29. Vaziri ND. Dyslipidemia of chronic renal failure: The nature, mechanisms, and potential consequences. Am J Physiol Renal Physiol 2006;290:F262-72.
30. Sugano M, Yamato H, Hayashi T, Ochiai H, Kakuchi J, Goto S, *et al.* High-fat diet in low-dose-streptozotocin-treated heminephrectomized rats induces all features of human type 2 diabetic nephropathy: A new rat model of diabetic nephropathy. Nutr Metab Cardiovasc Dis 2006;16:477-84.
31. Murea M, Freedman BI, Parks JS, Antinozzi PA, Elbein SC, Ma L. Lipotoxicity in diabetic nephropathy: The potential role of fatty acid oxidation. Clin J Am Soc Nephrol 2010;5:2373-9.
32. Marcovecchio ML, Dalton RN, Prevost AT, Acerini CL, Barrett TG, Cooper JD, *et al.* Prevalence of abnormal lipid profiles and the relationship with the development of microalbuminuria in adolescents with type 1 diabetes. Diabetes Care 2009;32:658-63.
33. Kern EF, Erhard P, Sun W, Genuth S, Weiss MF. Early urinary markers of diabetic kidney disease: A nested case-control study from the Diabetes Control and Complications Trial (DCCT). Am J Kidney Dis 2010;55:824-34.
34. Lee HS, Ku SK. Effect of Picrorrhiza rhizoma extracts on early diabetic nephropathy in streptozotocin-induced diabetic rats. J Med Food 2008;11:294-301.
35. Yokozawa T, Nakagawa T, Oya T, Okubo T, Juneja LR. Green tea polyphenols and dietary fibre protect against kidney damage in rats with diabetic nephropathy. J Pharm Pharmacol 2005;57:773-80.
36. Nakagawa T, Yokozawa T, Yamabe N, Rhyn DY, Goto H, Shimada Y, *et al.* Long-term treatment with Hachimi-jio-gan attenuates kidney damage in spontaneously diabetic WBN/Kob rats. J Pharm Pharmacol 2005;57:1205-12.
37. Nakagawa T, Yokozawa T, Terasawa K, Nakanishi K. Therapeutic usefulness of Keishi-bukuryo-gan for diabetic nephropathy. J Pharm Pharmacol 2003;55:219-27.
38. Makino H, Tanaka I, Mukoyama M, Sugawara A, Mori K, Muro S, *et al.* Prevention of diabetic nephropathy in rats by prostaglandin E receptor EP1-selective antagonist. J Am Soc Nephrol 2002;13:1757-65.
39. Kim HY, Kang KS, Yamabe N, Yokozawa T. Comparison of the effects of Korean ginseng and heat-processed Korean ginseng on diabetic oxidative stress. Am J Chin Med 2008;36:989-1004.
40. Kamalakkannan N, Stanely Mainzen Prince P. The influence of rutin on the extracellular matrix in streptozotocin-induced diabetic rat kidney. J Pharm Pharmacol 2006;58:1091-8.
41. Wang GG, Lu XH, Li W, Zhao X, Zhang C. Protective effects of luteolin on diabetic nephropathy in STZ-induced diabetic rats. Evid Based Complement Alternat Med 2011;2011:323171.
42. Qi MY, Kai-Chen, Liu HR, Su YH, Yu SQ. Protective effect of Icariin on the early stage of experimental diabetic nephropathy induced by streptozotocin via modulating transforming growth factor ß1 and type IV collagen expression in rats. J Ethnopharmacol 2011;138:731-6.
43. Cortes P, Mogensen CE. The Diabetic Kidney. Totowa, NJ: The Humana Press; 2006.

How to cite this article: Jain DP, Somani RS. Silibinin, a bioactive flavanone, prevents the progression of early diabetic nephropathy in experimental type-2 diabetic rats. Int J Green Pharm 2015;9:118-24.

Source of Support: The authors would like acknowledge Prof. M. N Nawale, President, Sinhgad Technical Education Society and Dr. K N Gujar, Principal, Sinhgad College of Pharmacy, Vadgaon (BK), Pune, India, for providing necessary facilities to carry out the present study, **Conflict of Interest:** None declared.