Effect of Silymarin on Streptozotocin-Nicotinamide—induced Type 2 Diabetic Nephropathy in Rats

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Introduction. Diabetic nephropathy is the major cause of end-stage renal disease worldwide. Silymarin is a flavonoid mixture obtained from *Silybum marianum.* Various preclinical and clinical studies have revealed that it has antidiabetic activity. The objective of this study was to evaluate the effect of silymarin on type 2 diabetic nephropathy in rats.

Materials and Methods. Non—insulin-dependent diabetes mellitus was induced in overnight-fasted male albino *Wistar* rats by an intramuscular injection of streptozotocin and nicotinamide. Eighteen rats with diabetic nephropathy and 6 rats without induced nephropathy were divided into 4 groups, each containing 6 animals. Group 1 was the normal control and group 2 was the DM control. Groups 3 and 4 were rats with diabetic nephropathy that received 60 mg/kg and 120 mg/kg of silymarin for 60 days.

Results. At the end of the study period, the diabetic control group had significantly higher blood glucose, glycosylated hemoglobin, urine volume, serum uric acid, and urine albumin levels when compared to the normal control group. Silymarin-treated groups showed significantly lower levels of blood glucose, glycosylated hemoglobin, urine volume, serum creatinine, serum uric acid, and urine albumin, when compared to the diabetic control group. Histopathological studies reports strongly supported the protective effect of silymarin.

Conclusions. These findings suggest that silymarin has protective effects for kidneys affected by type 2 diabetes mellitus. If the safety and efficacy is confirmed in humans, silymarin will be a good medication to prevent nephropathy-induced premature death in diabetic patients.

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INTRODUCTION

Diabetes mellitus (DM) is currently a major health problem all over the world and is a chronic metabolic disorder resulting from a variable interaction of hereditary and environmental factors. Diabetes mellitus is characterized by abnormal insulin secretion or insulin receptor or postreceptor events affecting metabolism involving the liver, kidney, and β cells of the pancreas.[1] It is classified into types 1 (insulin-dependent) and type 2 (non—insulin-dependent) DM. Type 2 DM is a heterogeneous disorder characterized by a progressive decline in insulin activity (insulin resistance), followed by the inability of β cells to compensate for insulin resistance (pancreatic β cell dysfunction).[2]

Diabetic nephropathy is an important

complication of both types of DM.[3] Diabetic nephropathy is the major cause of end-stage renal disease worldwide. The early changes in diabetic nephropathy are characterized by an increase in kidney size, glomerular volume, and kidney function followed by the accumulation of glomerular extracellular matrix, increased urinary albumin excretion, glomerular sclerosis, and tubular fibrosis. Last-stage overt diabetic nephropathy is clinically characterized by proteinuria, hypertension, and progressive renal insufficiency.[4] Diabetic nephropathy has been a growing threat in the world, and Eastern countries are not an exception. In Australia, type 2 DM patients starting dialysis increased 5-fold between 1993 and 2007, and in India, diabetic nephropathy is expected to develop in 6.6 million of the 30 million patients suffering from DM.[5-8]

Silymarin is a flavonoid mixture obtained from *Silybum marium*.[9] It is reported to have different properties including hepatoprotective, anti-inflammatory, antioxidant, and anti-cancer activities.[10] Previous studies have reported that silymarin has anti-DM activity in streptozotocin-treated DM (type 1) in male albino *Wistar* rats.[11] Various clinical studies revealed that silymarin has antidiabetic potential.[10,12,13] The objective of the present study was to evaluate the effect of silymarin on streptozotocin- and nicotinamide-induced type-2 diabetic nephropathy in male albino *Wistar* rats.

MATERIALS AND METHODS

Animals

Male albino *Wistar* rats (weight, 200 g to 220 g) obtained from Swamy Vivekanandha College of Pharmacy animal house were used for the study. They were maintained in standard laboratory conditions (temperature, 21 ± 2°C and relative humidity, 55% to 60%). They were fed with standard pellet diet and water ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee (SVCP/IAEC/M Pharm/01/Sep/2011) and the experiments were conducted in accordance with guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals in India.

Drugs and Pharmaceuticals

A gift sample of silymarin obtained from Micro Labs Ltd (Mumbai, India) was used for the study. The following chemicals were purchased from various pharmaceutical companies: streptozotocin (Sisco Research Laboratories Pvt Ltd, Mumbai, India), nicotinamide (Loba Chemicals Pvt Ltd, Mumbai, India), citric acid (Loba Chemicals Pvt Ltd, Mumbai, India), sodium citrate (Loba Chemicals Pvt Ltd, Mumbai, India), carboxyl methyl cellulose (Loba Chemicals Pvt Ltd, Mumbai, India), formaldehyde (Nice Chemicals Pvt Ltd, Mumbai, India), sodium phosphate monobasic (Loba Chemicals Pvt Ltd, Mumbai, India), and sodium phosphate dibasic (Loba chemical Pvt. Ltd., Mumbai).

Induction of Type 2 Diabetes Mellitus

Twenty-four male albino *Wistar* rats were divided into 4 groups, each containing 6 animals. Group 1 was the normal control; group 2, DM control; and groups 3 and 4, DM + low-dose and high-dose silymarin (60 mg/kg and 120 mg/kg, respectively). Streptozotocin was freshly dissolved in citrate buffer (0.1 M; pH, 4.5), and nicotinamide was dissolved in normal physiological saline and maintained on ice prior to use. Non—insulin-dependent DM was induced in overnight-fasted rats by an intramuscular injection of 60 mg/kg streptozotocin, and thereafter, 120 mg/kg nicotinamide was injected after 5 minutes. The elevated plasma glucose was determined after 3 days of streptozotocin and nicotinamide administration and those rats with fasting glucose levels greater than 250 mg/dL were served as diabetic rats and used in the study.[14]

Sample Collection and Biochemical Assays

After 60 days of silymarin treatment, the animals were kept for 24 hours in metabolic cage (Instruments & Chemicals Pvt Ltd, Ambalacity, India) for urine collection. Volume of urine noted and the samples were used to analyse the urine creatinine, urea, uric acid, and albumin levels. Finally, blood samples were collected through cardiac puncture. The blood samples were used to analyse the blood glucose, glycosylated hemoglobin, creatinine, urea, and uric acid levels. The urine and blood parameters were evaluated using a semi-auto-analyser (Mind Rays Ba-88a, Mindray Medical India Pvt Ltd, Mumbai, India) following suitable methods.

Histopathological Studies

The animals were sacrificed by cervical dislocation, and the kidneys were dissected out and weighed. Then, the kidneys were stored in 10% buffered formaline solution (formaldehyde, 100 mL; sodium phosphate monobasic, 4 g; sodium phosphate dibasic, 6.5 g; and water, 900 mL) and subjected to further processing for histopathological evaluation.

Statistical Analysis

The values of continuous variables were expressed as mean ± standard error. The 1-way analysis of variance, followed by the Tukey multiple comparison test, was used to analyse the effect of different doses of silymarin when compared to control, with the help of GraphPad Instat software (version 3.01, GraphPad Software Inc, La Jolla, USA). A *P* value less than .05 was considered significant.

RESULTS

Effect of Silymarin on Body and Kidney Weights

After 60 days of treatment, body weight of the rats in the normal control group was significantly increased when compared to initial body weight ($P < .001$). However, the body weight of the rats in the DM control group significantly decreased when compared to the initial body weight ($P < .001$). Diabetic rats treated with low-dose silymarin (60 mg/kg) did not show any significant changes when compared to the initial body weight. However, the diabetic rats treated with high-dose silymarin (120 mg/kg) showed significant increase when compared to the initial body weight ($P < .01$; Figure 1).

In the DM control group, the kidney weights significantly ($P < .001$) increased when compared to the normal control group. In the diabetic rats treated with low-dose silymarin ($P < .001$) and low-dose silymarin ($P < .001$), the kidney weight was significantly reduced when compared to the DM control group (Figure 2).

Effect of Silymarin on Blood Parameters

Blood Glucose Levels. In the diabetic control group, fasting blood glucose level was increased significantly ($P < .01$) after 60 days of treatment when compared to the initial blood glucose level. In the diabetic rats treated with low-dose and high-dose silymarin, the blood glucose levels were significantly lower when compared to initial blood glucose level ($P < .01$ and $P < .001$, respectively; Figure 3).

Glycosylated Hemoglobin Level. In the diabetic control group, glycosylated hemoglobin levels were significantly increased when compared to the normal control group ($P < .001$). The diabetic rats treated with high-dose silymarin showed a significant decrease in glycosylated hemoglobin level when compared to diabetic control group ($P < .001$; Table 1).

Serum Creatinine Level. In the diabetic control

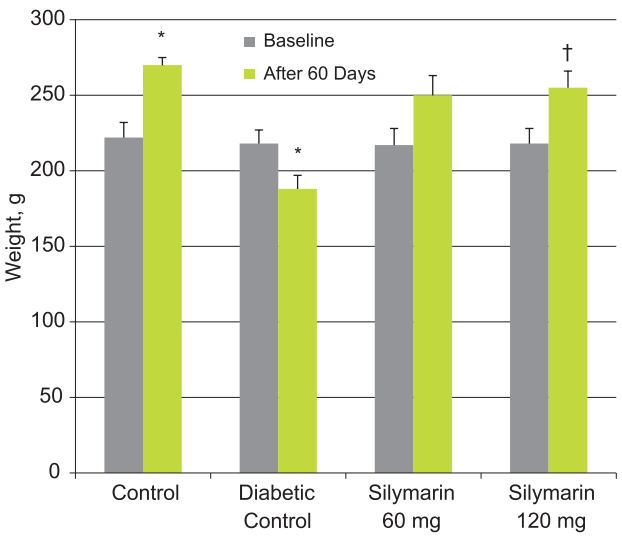

Figure 1. The effect of silymarin on body weight in rats.
*$P < .01$
†$P < .001$ when compared to initial body weights (unpaired *t* test)

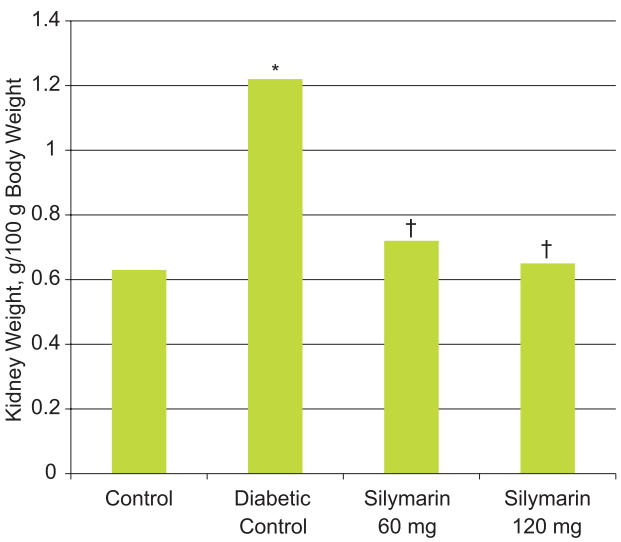

Figure 2. The effect of silymarin on kidney weight in rats.
*$P < .001$ when compared to normal control group
†$P < .001$ when compared to diabetic control group

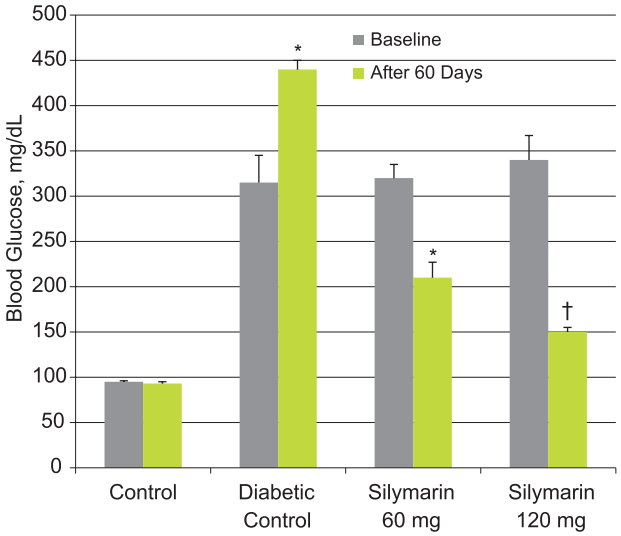

Figure 3. The effect of silymarin on blood glucose levels in rats.
†$P < .001$
*$P < .01$ when compared to initial blood glucose levels (unpaired *t* test)

group, serum creatinine levels were increased but did not show any significant change when compared to the normal control group. In the diabetic rats treated with low-dose and high-dose silymarin, significantly lower serum creatinine levels were observed when compared to the diabetic control group ($P < .01$ and $P < .05$, respectively; Table 1).

Serum Uric Acid Level. In the diabetic control group, serum uric acid levels were significantly increased when compared to the normal control group ($P < .05$). In the diabetic rats treated with low-dose and high-dose silymarin, significantly lower serum uric acid levels were observed when compared to the diabetic control group ($P < .05$; Table 1).

Effect of Silymarin on Urine Parameters

Urine Volume. In the diabetic control group, urine volume was significantly increased when compared to the normal control group ($P < .001$). In the diabetic rats treated with low-dose and high-dose silymarin, a significantly lower urine volume was observed when compared to the diabetic control group ($P < .001$; Table 2).

Urine Albumin Level. In the diabetic control group, urine albumin level was significantly increased when compared to the normal control group ($P < .001$). In the diabetic rats treated with low-dose and high-dose silymarin, a significantly lower urine albumin level was observed when compared to the diabetic control group ($P < .01$ and $P < .001$, respectively; Table 2).

Histopathological Studies

The histopathological studies revealed that the control group rats showed normal kidney tubules with healthy epithelial cells. The diabetic control group rats showed disrupted tubules, degeneration and necrosis of epithelial cells, and intertubular hemorrhage. In the treatment group of low-dose

Table 1. Effect of Silymarin on Blood Parameters in Rats

		Diabetic Rats		
Parameter	Normal Controls	Control	Silymarin (60 mg/kg)	Silymarin (120 mg/kg)
Glycosylated hemoglobin, %	5.14 ± 0.20	14.18 ± 0.39*	12.26 ± 0.98	9.88 ± 1.10†
Serum creatinine, mg/dL	0.49 ± 0.02	0.59 ± 0.06	0.36 ± 0.03†	0.43 ± 0.02‡
Blood urea, mg/dL	38.55 ± 1.50	41.85 ± 1.31	52.73 ± 5.15	43.16 ± 4.49
Blood uric acid, mg/dL	1.01 ± 0.19	1.61 ± 0.16§	1.05 ± 0.10‡	0.93 ± 0.09‡

*$P < .001$
†$P < .01$
‡$P < .01$ when compared to normal control group
§$P < .05$ when compared to normal control group

Table 2. Effect of Silymarin on Urine Parameters in Rats

		Diabetic Rats		
Parameter	Normal Controls	Control	Silymarin (60 mg/kg)	Silymarin (120 mg/kg)
Urine volume, mL	1.93 ± 0.277	19.88 ± 1.244*	11.50 ± 1.065†	9.58 ± 0.7791†
Urine creatinine, mg/dL	66.76 ± 2.897	35.41 ± 1.849*	37.15 ± 1.958	32.56 ± 2.009
Urine urea, mg/dL	83.01 ± 1.585†	90.06 ± 1.176	97.91 ± 2.887	97.93 ± 2.134‡
Urine uric acid, mg/dL	24.33 ± 0.864†	11.73 ± 1.053*	8.60 ± 1.154	11.61 ± 1.266†

*$P < .001$ when compared to normal control group
†$P < .001$
‡$P < .01$ when compared to diabetic control group

silymarin, regenerating tubular epithelium and moderate intertubular hemorrhage were seen. Diabetic rats treated with high-dose silymarin showed kidneys depicting normal tubules with intact epithelium and presence of few erythrocytes between tubules.

DISCUSSION

Silymarin is a mixture of flavonolignans from the fruits of *Silybum marianum.* It contains silybin, isosilybin, silydianin, and silychristin.[15] It has been approved as a safe herbal product for renal protection in high doses. It has antioxidant effects via increase of gene expression of antioxidant enzymes and a number of the most important protection mechanisms against free radicals damage containing superoxide dismutase, glutathione peroxidase, and catalase.[16]

The results of our study showed that in silymarin-treated diabetic rats, body weights were increased significantly probably due to the protective effect of silymarin in controlling muscle wasting, ie, reversal of gluconeogenesis.[17] However, in the diabetic control group, body weights decreased significantly due to increasing muscle wasting.[18] The kidney weight of the diabetic rats increased significantly due to renal enlargement, which is one of the key features occurring during initial changes by DM. In earlier stages of diabetic nephropathy, hypertrophy and hyperfunctioning of the kidneys with a typical increase in kidney size and glomerular filtration rate is observed.[19] In the silymarin-treated group of rats, kidneys weight were significantly lower when compared to the diabetic control group.

Our study revealed that in the diabetic control group of rats, the volume of urine was increased significantly. This can be explained by the fact that the renal tubules are unable to absorb all of the glucose filtered in the glomeruli. The renal excretion of glucose requires excretion of water and produces an osmotic diuresis. This diuresis is called polyuria or excessive urination. It can cause dehydration, resulting in dry skin and blurred vision, which is due to fluctuation in the amount of glucose and water in the lenses of the eye during dehydration. Glucose needs water to flow from the body. Loss of water causes an increase in the serum polarity that stimulates the thirst center in the hypothalamus.[20] Nonetheless, in the silymarin-treated group of rats, urine volume was significantly lower. Urine creatinine, urea, and uric acid levels did not show any significant change in the silymarin groups. However, urine albumin level increased in the diabetic control group due to proteins from the kidney that appear in the urine as a consequence of normal process of cell turnover and metabolism. The release of these proteins was enhanced during the kidney function impairment as happens in DM.[21] However, in the silymarin-treated group, release of protein in urine significantly decreased.

Our study showed that the blood glucose of diabetic control rats was significantly increased, but in treatment group, it was significantly decreased when compared to initial blood glucose level. Our results are consistent with the results of Jadhav and colleagues who reported that silymarin showed a significant decrease in the average fasting blood glucose level in drug-treated groups when compared to a control group with type 2 DM.[22] In the diabetic control group, glycosylated hemoglobin was significantly increased, but in the treatment group, it was significantly decreased. Our results were consistent with the results of Abenavoli and colleagues, who reported that there was a significant decrease in glycosylated hemoglobin levels in the silymarin-treated group (120 mg/kg) when compared to the diabetic control group.[23] The reaction between glucose and hemoglobin is the production of glucose with the free amino groups of the N-terminals of the b-chain of the hemoglobin molecules. The process is slow, continuous, and irreversible. It serves as an indicator of metabolic control in DM.[24] Each 1% reduction in glycosylated hemoglobin is associated with a 37% reduction in microvascular complications, 18% fewer myocardial infarction, and 21% fewer DM-related deaths.[25] In our diabetic control group, serum creatinine level increased, but did not show any significant change. In the treatment group, it significantly decreased. The increase of serum creatinine in DM is due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume.[26] In the diabetic control group, serum uric acid level significantly increased, but in the treatment group, it significantly decreased. The increase in uric acid level could be due to the fact that filtered uric acid is both reabsorbed and excreted in the proximal tubule through a voltage-sensitive urate

channel and a urate-anion exchange mechanism. Hyperuricemia can be a result of either increased production or decreased excretion.[27] Results of histopathological studies strongly support the outcome of our study.

CONCLUSIONS

In this preliminary investigation, elevation of blood glucose, glycosylated hemoglobin, serum creatinine, serum uric acid, and urine albumin levels in the diabetic control group was significantly reversed by silymarin in streptozotocin- and nicotinamide-induced type 2 diabetic nephropathy in male albino *Wistar* rats. Therefore, our study suggested that silymarin may be used as a renal protective for humans affected long-term by type 2 DM, after confirming its efficacy and safety in well-controlled clinical trials. If it is confirmed in humans, silymarin may be a good medication to prevent nephropathy-induced premature death in diabetic patients.

CONFLICT OF INTEREST

None declared.

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