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### Research Article

## Protective Effect of Diosgenin on Streptozotocin-induced Diabetic Nephropathy in Experimental Rats

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### ABSTRACT

Diabetes mellitus (DM) is a multifactorial metabolic disorder associated with diabetes-related vascular diseases. Oxidative stress, along with inflammation, is the key factor leading to diabetic complications. The present study was designed to investigate the protective role of diosgenin, a steroidal saponin, in diabetes-induced early kidney injury, oxidative stress markers, and histopathological changes in kidney of diabetic rats, induced by single intraperitoneal injection of streptozotocin 55 mg/kg weight (b.w.). After 72 hours, experimental rats received diosgenin at different doses (10, 20, and 40 mg/kg b.w.) once daily for four weeks. At the end of the experiment, diabetic rats showed a significant increase in the levels of plasma glucose, glycosylated hemoglobin with a significant decrease in insulin and total hemoglobin. The activities of antioxidant enzymes such as superoxide dismutase, catalase, reduced glutathione, and the levels of reduced glutathione were decreased while increases in the levels of lipid peroxidation markers were observed in kidney tissues of diabetic rats. Oral administration of diosgenin to diabetic rats considerably shrivelled the plasma glucose and exaggerated the endocrine level supported a dose dependent manner. Diosgenin at a dose of 40 mg/kg b.w. was more pronounced effect than the other two doses and used for further studies. All the manifestations observed in diabetic rats were significantly reversed to near normal at a dose of 40 mg/kg b.w. of diosgenin. These findings recommend that diosgenin may have a helpful role against excretory organ harm evoked by aerobic stress within the diabetic state.

### INTRODUCTION

Diabetes Mellitus (DM) could be a chronic disorder characterized by hyperglycemia with distraction in saccharide, fat, and macromolecule metabolism.<sup>[1]</sup> Patients with DM typically have poor glycemic management and develop various microvascular complications, together with nephrosis.<sup>[1-3]</sup> Diabetic nephrosis, a condition of progressive harm to the urinary organ, is qualified by thickening of capillary basement membrane, glomerulosclerosis, capillary hypertrophy, podocyte loss, mesangial cells enlargement, and tubulointerstitial pathology.<sup>[4]</sup> Diabetic nephrosis is related to persistently increased albuminuria, declined capillary filtration rate, fluid retention, and increased blood pressure (BP).<sup>[2,3]</sup> The precise cause for diabetic nephrosis remains mysterious; however, the structural and useful

alterations of the urinary organ occur principally because of chronic hyperglycaemia and protracted cardiovascular disease.<sup>[5-6]</sup> Lipemia has been urged a freelance risk issue and highly determinant of the development of nephrosis in patients with DM.<sup>[7]</sup> Studies have urged that elevation in current lipids could promote excretory organ illness progression.<sup>[7]</sup>

Phytopharmaceuticals square measure gaining importance in medical aid also as ancient medication causes of its non-addictive and non-toxic nature.<sup>[8]</sup> Diosgenin has a present aglycone of steroid glucoside found extravagantly in legume and yam like plant genus species. It is a widely known precursor or of varied artificial internal secretion medication that square measure extensively utilized in the pharmaceutical trade.<sup>[9,10]</sup> Diosgenin could be pharmacologically evaluated for its glucose-lowering

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effect,<sup>[11]</sup> antioxidant, and hyperlipidemic activity,<sup>[3]</sup> antilipoperoxidative,<sup>[12]</sup> anti-inflammatory.<sup>[13]</sup> Moreover, diosgenin was a rich source in food as an anti-diabetic activity in the present experimental models.<sup>[11,14]</sup> This report was additionally reinforced by the very fact that many viscous rate-limiting enzymes usually concerned in glucose metabolism altered within the diabetic condition were protected by diosgenin.<sup>[15,16]</sup> Whereas there's ample proof implying that diosgenin is major in foods could also be useful as another medication to treat polygenic disease, and with their related complications, a lot of experimental studies square measure guaranteed to handle if the protective role of diosgenin in the treatment of diabetic-related complications. Visible of this light-weight, this study is initiated to show the impact of diosgenin on streptozotocin evoked diabetic nephritis in experimental rats.

## MATERIALS AND METHODS

### Animals

All animal experiments were conducted in accordance with The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) tips. The Institutional Animal Ethical Committee approved the study protocol of R. C. Patel Institute of Pharmaceutical Education and Research (RCPIPER), Shirpur (Approval No. IAEC/RCPIPER/2018-19/02), Shirpur. Adult male anomaly rats of Wistar strain (180–220 g) were obtained from the College Animal House of RCPIPER, Shirpur, Dhule, India. The rats were acclimatized under standard laboratory conditions. They were housed in polypropylene cages maintained at  $25 \pm 1^\circ\text{C}$  and 12 hours light-dark cycle. They had free access to a standard pellet diet (Pranav Agro-Industries Ltd., Sangli, India) and potable *ad libitum*.

### Drugs and Chemicals

Diosgenin and streptozotocin were obtained from Sigma Aldrich (USA). Metformin was obtained from the local pharmacy. Plasma creatinine, blood urea nitrogen (BUN), serum insulin, albumin, total cholesterol, triglyceride, and enzyme-linked immunosorbent assay (ELISA) Kits for TNF- $\alpha$  (Cas No. 88-7324), and IL-6 (Cas No. 88-7064) kits were supplied by Accurex biomedical Pvt. Ltd. (Mumbai, India).

### Induction of Diabetes in Experimental Rats

The animal model of diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg b.w.) dissolved in 0.2 mL of 0.1 M citrate buffer, pH 4.5. Control rats were injected with the vehicle (0.2 mL of 0.1 M citrate buffer, pH 4.5) alone. Animals were given a 5% glucose solution rather than drinking water for 2 days to overcome the hypoglycaemic coma that happens within the first 24 hours following streptozotocin injection. Rats were screened for blood sugar levels 48 hours when

STZ blood samples were withdrawn from the lateral tail vein, and glucose concentration was measured from overnight fasted animals 10–12 hours. Rats having glucose concentration exceeding 200 mg/dL were considered diabetic and included within the experiment.<sup>[17]</sup> When 72 hours of streptozotocin injection, oral administration of diosgenin was followed once daily for four weeks.<sup>[18]</sup>

### Experimental Design

The animals were divided into six groups ( $n = 6$ ), a total of 36 rats (30 surviving diabetic rats, 6 normal control rats) were used. Metformin 70 mg/kg was used for standard control. Diosgenin was dissolved in-vehicle solution (carboxymethyl cellulose), and different doses of diosgenin were administered orally once a daily for four weeks.<sup>[19]</sup>

*Group I:* normal management rats (vehicle-treated).

*Group II:* normal rats + diabetic management rats.

*Group III:* diabetic control rats + metformin (70 mg/kg)

*Group IV:* diabetic control rats + diosgenin (10 mg/kg b.w.).

*Group V:* diabetic control rats + diosgenin (20 mg/kg b.w.).

*Group VI:* diabetic control rats + diosgenin (40 mg/kg b.w.).

After 4 weeks of treatment, rats fasted overnight, and blood samples were collected and analyzed for the estimation of various biochemical parameters in plasma. Rats were separately placed in a metabolic cage, twenty-four-hour total urine volume was measured, and the same was used for estimation of renal function. Rats were sacrificed, and kidneys were collected to study oxidative stress as well as histopathological observations.

### Bodyweight, Kidney Weight, and Excretory Organ Index

At the end of treatment, body and kidney weight were measured by gravimetric methodology using an electronic weighing machine, and kidney index<sup>[20]</sup> was calculated.

### Estimations of Plasma Biochemical Parameters

Creatinine,<sup>[21]</sup> urea nitrogen,<sup>[22]</sup> plasma glucose and albumin<sup>[23]</sup> were determined using commercial diagnostic kits.

### Evaluation of Oxidative Stress

A right kidney of an individual rat was isolated, washed in cold saline and prepared 100% w/v homogenate using 0.15 M KCl by centrifuging at 10,500 g for 10 minutes at  $4^\circ\text{C}$ . The supernatant obtained was used for the estimation of lipid peroxidation (MDA)<sup>[14]</sup> and catalase (CAT).<sup>[24]</sup> Homogenate was further centrifuged at 1,000 g for 20 min at  $4^\circ\text{C}$ , and the supernatant was used for estimation of superoxide dismutase (SOD)<sup>[25]</sup> and glutathione (GSH).<sup>[26]</sup> Protein concentrations of homogenates were determined, according to Lowry *et al.*<sup>[27]</sup>

### Estimation of Cytokines

A 10% kidney homogenate was prepared in ice-chilled phosphate buffer (50 mM pH 7.4) the homogenate was



centrifuged at 2000 g for 20 min at 4°C and supernatant was used for the estimation of TNF- $\alpha$  and IL-6 Using ELISA kits.<sup>[28]</sup>

### Histopathological Studies:

A left kidney of individual rat stored in 10% formalin solution was embedded with paraffin. For histopathological feature examination, 5  $\mu$ m sections were stained with hematoxylin and eosin for the examination using a light microscope.

### Statistical Analysis

All the data were expressed as mean  $\pm$  SEM. Data analysis performed by using Graph Pad Prism 8.0 software. The statistical analyses were conducted with the help of analysis of variance (ANOVA) followed by Dunnett's test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  were considered to be statistically significant as compared to diabetic group. ### $p < 0.001$  was considered to be statistically significant when compared to normal.

## RESULT AND DISCUSSION

### Physiological Parameters Body Weight and Kidney Weight

The diabetic rats exhibited a significant (###  $p < 0.001$ ) decrease in body weight. The treatment of diosgenin at 40 mg/kg was able to control the weight loss significantly (\*\*\* $p < 0.001$ ), the kidney weight was significantly ( $p < 0.001$ ) increased in the diabetic group were observed when compared to normal control rats. The treatment of Diosgenin (40 mg/kg) was significantly decreased ( $p < 0.01$ ) when compared to diabetic control rats (Table 1).

**Table 1:** Effect of diosgenin on the body, kidney weight, and kidney index.

Groups	Body weight (g)	Kidney weight (g)
Control	201 $\pm$ 1.5	1.100 $\pm$ 0.10
Diabetic	164 $\pm$ 10 <sup>##</sup>	2.078 $\pm$ 0.19 <sup>##</sup>
Metformin 70 mg	185 $\pm$ 1.2 <sup>*</sup>	1.437 $\pm$ 0.10 <sup>**</sup>
Diosgenin 10 mg/kg	180 $\pm$ 7.8 <sup>**</sup>	1.525 $\pm$ 0.14 <sup>*</sup>
Diosgenin 20 mg/kg	184 $\pm$ 2.6 <sup>**</sup>	1.387 $\pm$ 0.05 <sup>**</sup>
Diosgenin 40 mg/kg	184 $\pm$ 1.1 <sup>*</sup>	1.205 $\pm$ 0.08 <sup>***</sup>

Data was expressed as mean  $\pm$  SEM  $n = 6$  and analyzed by one way ANOVA followed by Dunnet test for each parameter separately.  $p < 0.0001$  = significant<sup>\*\*\*</sup>,  $p < 0.01$  = very significant<sup>\*\*</sup>,  $p < 0.05$  = significant<sup>\*</sup> as compared to DM group and # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  as compared to DM group. ( $n = 6$ ) as compared to normal group ( $n = 6$ )

**Table 2:** Effect of diosgenin on lipid profile.

Groups	Cholesterol(mg/dL)	Triglyceride (mg/dL)
Normal	79.15 $\pm$ 22.05	88.37 $\pm$ 4.5
Diabetic	140.60 $\pm$ 8.62 <sup>###</sup>	141.05 $\pm$ 9.96 <sup>##</sup>
Metformin 70 mg/kg	93.32 $\pm$ 13.89 <sup>*</sup>	103.1 $\pm$ 7.63 <sup>*</sup>
Diosgenin 10 mg/kg	84.39 $\pm$ 9.43 <sup>*</sup>	100.2 $\pm$ 7.27 <sup>*</sup>
Diosgenin 20 mg/kg	80.34 $\pm$ 8.01 <sup>**</sup>	93.48 $\pm$ 16.1 <sup>**</sup>
Diosgenin 40 mg/kg	77.87 $\pm$ 4.73 <sup>**</sup>	92.19 $\pm$ 4.54 <sup>**</sup>

Values are expressed as mean  $\pm$  SEM  $n = 6$  Data was analyzed by one way ANOVA followed by Dunnet test for each parameter separately.  $p < 0.0001$  = significant<sup>\*\*\*</sup>,  $p < 0.01$  = very significant<sup>\*\*</sup>,  $p < 0.05$  = significant<sup>\*</sup> as compared to normal group and # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  as compared to DM group. ( $n = 6$ )

### Lipid Profile

The diabetic group showed a significant increase in total cholesterol and triglycerides levels when compared to the normal control. Treatment with metformin and diosgenin (20 and 40 mg/kg) significantly decreased total cholesterol and triglyceride levels as compared to the diabetic group (Table 2).

### Biochemical Parameters

Diabetic group exhibited significant (### $p < 0.001$ ) elevation in blood glucose level when compared to normal control. This multi-fold increase in blood glucose suggests severe induction of diabetes in the animals. Metformin group (positive control) significantly (\*\*\* $p < 0.001$ ) reduced the blood glucose level. Diosgenin showed a significant blood glucose-lowering activity at 40 mg/kg dose level (\*\*\* $p < 0.001$ ) but not enough to restore it to the normal levels.

After 4 weeks, significant ( $p < 0.001$ ) increase in 24 hours. Total urine volume, albumin, serum creatinine, and urea nitrogen were observed in the diabetic rats. Diosgenin (40 mg/kg) treatment for four weeks significantly ( $p < 0.001$ ) prevented the rise in 24 hours total urine volume as well as albumin level and also serum creatinine and urea nitrogen (Table 3).

### Renal Hypertrophy Index

The kidney weight to body weight ratio in the diabetic group was increased significantly (### $p < 0.001$ ) as compared to the control. metformin (\*\*\* $p < 0.001$ ) and diosgenin at 40 mg/kg showed a significant decrease (\*\*\* $p < 0.001$ ) in kidney weight to body weight ratio indicating decreased renal hypertrophy index (Fig. 1).

**Table 3:** Effect of diosgenin on biomarkers of renal damage.

Groups	Blood glucose mg/dL	Urine albumin (mg/dL)	Urine volume (mL)	Urea nitrogen (mg/dL)	Serum creatinine (mg/dL)
Control	93.50 ± 1.64	2.6 ± 0.35	2.59 ± 0.34	1.15 ± 0.08	0.62 ± 0.14
Diabetic	245.2 ± 9.37###	4.9 ± 0.30##	4.93 ± 0.30##	3.40 ± 0.22##	1.46 ± 0.32##
Metformin 70 mg/kg	204.3 ± 23.59*	3.0 ± 0.33**	3.01 ± 0.33**	2.35 ± 0.18***	0.74 ± 0.13*
Diosgenin 10 mg/kg	189.6 ± 41.48*	3.3 ± 0.23*	0.31 ± 0.22*	1.97 ± 0.14***	0.74 ± 0.11*
Diosgenin 20 mg/kg	185.7 ± 51.11*	3.0 ± 0.25**	0.30 ± 0.54**	2.16 ± 0.03***	0.62 ± 0.17**
Diosgenin 40 mg/kg	167.6 ± 63.82**	2.7 ± 0.25***	2.70 ± 0.25**	1.23 ± 0.26***	0.59 ± 0.07**

Data was expressed as mean ± SEM n = 6 and analyzed by one way ANOVA followed by Dunnet test for each parameter separately. p < 0.0001 = significant \*\*\*, p < 0.01 = very significant \*\*, p < 0.05 = significant \* as compared to DM group and #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to DM group. (n = 6) as compared to normal group (n = 6).

### Kidney Antioxidants Parameters

The results of the kidney antioxidants parameters was depicted in Fig. 2.

### Cytokine Estimation

The results of the cytokine estimation was depicted in Fig. 3.

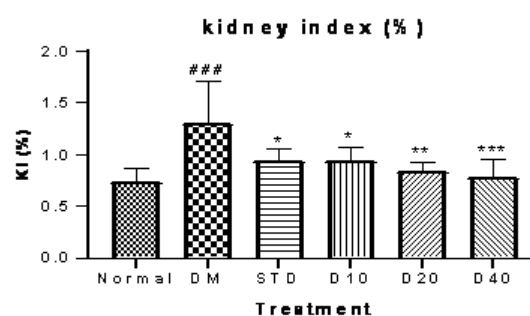
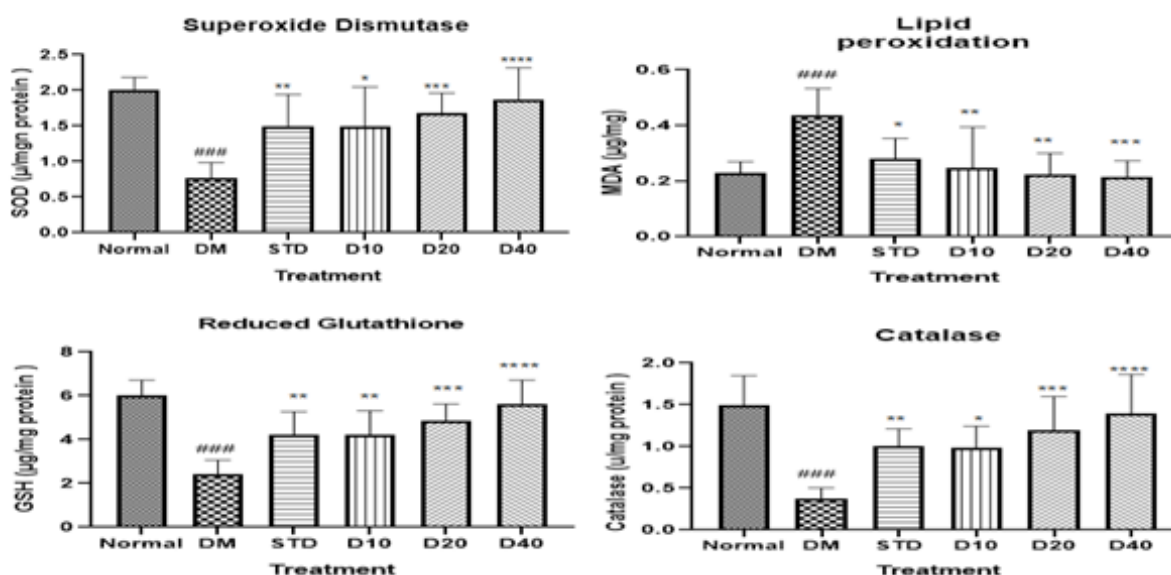
### Histopathology

Histological changes and renal damage investigated by H&E staining with 10 magnification showed severe morphological changes such as vacuolar degeneration, tubular collapse, and hyalinization of arterioles and scarring of tissues in the diabetic group. In contrast, diosgenin treatment (40 mg/kg) significantly preserved the architectural structure of the kidney and protected it from diabetes-associated injury (Fig. 4).

Data was expressed as mean ± SEM and analyzed by one way ANOVA followed by Dunnet test for each parameter separately. P < 0.0001 = significant \*\*\*, P < 0.01 = very significant \*\*, P < 0.05 = significant \* as compared to DM group and #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to DM group. (n = 6) as compared to normal group (n = 6).

Data was expressed as mean ± SEM and analyzed by one way ANOVA followed by Dunnet test for each parameter separately. P < 0.0001 = significant \*\*\*, P < 0.01 = very significant \*\*, P < 0.05 = significant \* as compared to sDM group and #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to DM group. (n = 6) as compared to normal group (n = 6).

Data was expressed as mean ± SEM and analyzed by one way ANOVA followed by Dunnet test for each parameter

**Fig. 1:** Effect of diosgenin on kidney hypertrophy index.**Fig. 2:** Effect of diosgenin in superoxide dismutase (A), lipid peroxidation (B), reduced glutathione activity (C), catalase (D).



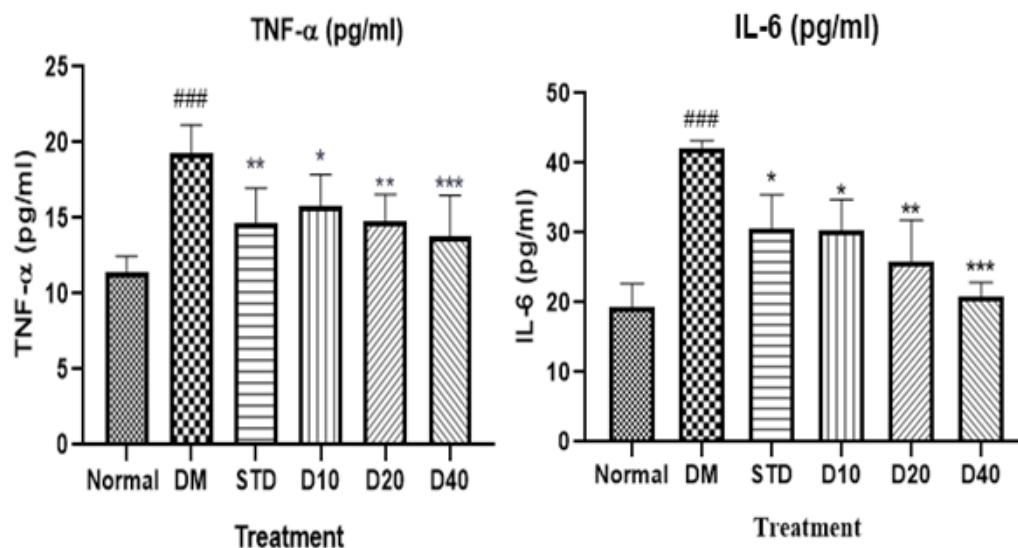


Fig. 3: Effect of diosgenin on cytokine estimation.

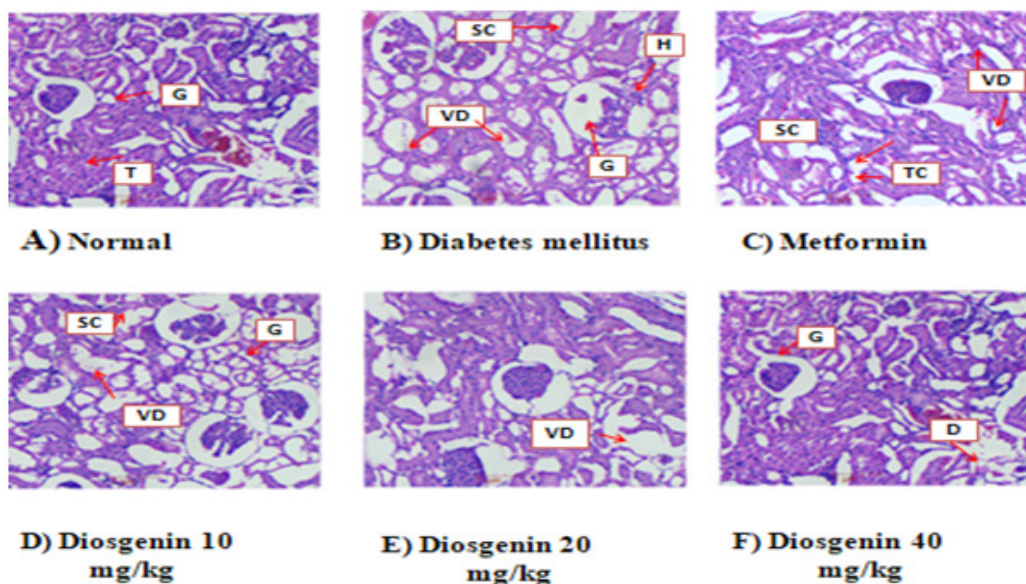


Fig. 4: Photomicrographs of histopathological analysis of kidney by H&amp;E staining;

(A) control, (B) diabetic, (C) metformin groups. (D, E, and F) 10, 20, and 40 mg/kg diosgenin, respectively. D = Degeneration of PCT; VD = Vacuolar degeneration; SC = Scarring of tubule; G = glomeruli; H = Hyalinisation of vessels; and TC = tubular collapse.

separately.  $P < 0.0001$  = significant<sup>\*\*\*</sup>,  $P < 0.01$  = very significant<sup>\*\*</sup>,  $P < 0.05$  = significant<sup>\*</sup> as compared to DM group and  $\#p < 0.05$ ,  $\#\#p < 0.01$ ,  $\#\#\#p < 0.001$  as compared to DM group. ( $n=6$ ) as compared to normal group ( $n=6$ ).

## DISCUSSION

In the present study, the obtained results from the study suggest that diosgenin protects against the development of diabetic nephropathy by preventing oxidative stress and inhibiting inflammatory mediators like TNF- $\alpha$  and IL-6, our results show that loss in body weight in diabetic condition occurs due to excessive breakdown of muscle tissue, oxidation of proteins and gluconeogenesis,

the treatment of diosgenin at 40 mg/kg was able to control the weight loss significantly, the kidney weight was significantly increased in the diabetic group were observed when compared to normal control rats. The treatment of diosgenin (40 mg/kg) was significantly decreased when compared to diabetic control rats. Chronic hyperglycemia is associated with impaired lipid metabolism due to abnormal insulin regulation or secretion. Insulin deficiency induces the mobilization of free fatty acids from peripheral fat deposits causing high levels of serum lipids. Insulin activates lipoprotein lipase, which hydrolyzes triglycerides. Insulin deficiency thus results in hypertriglyceridemia, a condition generally observed in diabetes.<sup>[28,29]</sup> The treatment with diosgenin

resulted in normalization of diabetic dyslipidemia which may contribute to the renal protective effect. Treatment with metformin and diosgenin (20 and 40 mg/kg) significantly decreased total cholesterol and triglyceride levels as compared to diabetic group.

Renal hypertrophy is an important pathological manifestation of diabetes-induced early kidney injury<sup>[30]</sup> and the effectiveness of diosgenin was determined by its ability to control the hypertrophy, diosgenin at 40 mg/kg showed a significant decrease in kidney weight to body weight ratio indicating decreased renal hypertrophy index.

The diabetic group demonstrated a significant decrease in renal endogenous antioxidant enzymes. Treatment with diosgenin at 20 and 40 mg/kg and metformin significantly restored the activity of SOD and CAT as compared with the diabetic group. Reduced glutathione is an important endogenous antioxidant that is considered as the major regulator of intracellular redox status. Diminished regeneration of GSH accelerates the oxidative stress during diabetes.<sup>[30]</sup> The level of GSH in the diabetic group was reduced significantly compared to control. Treatment with diosgenin at 20 and 40 mg/kg and metformin increased GSH levels as compared to the diabetic group. The above data portrays the positive effect of diosgenin on the antioxidant defense system of the kidney.

Kidney lipid peroxidation in lipid peroxidation is believed to be a decisive indicator of oxidative stress. It is produced due to the exaggerated generation of ROS from the mitochondrial electron transport chain and inflammatory response.<sup>[31]</sup> The renal tissue lipid peroxidation level represented as MDA levels were increased significantly in the diabetic group as compared to control. Diosgenin treatment at 40 mg/kg and metformin successfully ameliorated lipid peroxidation as compared to the diabetic group. Treatment with diosgenin augmented the intracellular antioxidant defense and was found to be effective in controlling the oxidative stress under hyperglycaemic conditions.

Oxidative stress is known to regulate the expression of several genes that are concerned in the production of inflammatory cytokines, including TNF- $\alpha$  to initiate the proliferative responses. The accumulation of perivascular inflammatory cell infiltrates like neutrophils contributes more to reactive oxygen species production that triggers the destruction of lung tissues and promotes intravascular platelet aggregation. Moreover, the lack of the available NO activates the inflammatory and the proliferative cascades in pH. The diabetic group showed significantly increased tumor necrosis factor (TNF- $\alpha$ ) when as compared to the normal control. Treatment with metformin and diosgenin (20 and 40 mg/kg) significantly decreased of tumor necrosis factor (TNF- $\alpha$ ) as compared to the diabetic group. Thus, treatment with diosgenin resulted in normalization of the production of inflammatory cytokines, as well as TNF- $\alpha$ , which may contribute to a renal protecting effect. Similarly, the diabetic group also showed a significantly

increased interleukin-6 (IL-6) once as compared to normal control. Treatment with diosgenin (20 and 40 mg/kg) significantly decreased interleukin-6 (IL-6) as compared to diabetic cluster and treatment of metformin not much more significant than 10 and 20 mg/kg of diosgenin.

### Histopathological Analysis

Histological analysis of the kidney was carried out to understand whether these biochemical modifications yielded structural changes at the microscopic level. Histological changes and renal damage, investigated by A & E staining with 10 magnification, showed severe morphological changes such as vacuolar degeneration, tubular collapse and hyalinization of arterioles, and scarring of tissues in the diabetic group. In contrast, diosgenin treatment (40 mg/kg) significantly preserved the architectural structure of the kidney and protected it from diabetes-associated injury (Fig. 4). These histological findings are following the above biochemical results, wherein diosgenin efficiently reduced the damage to the kidney in a dose-dependent manner. The thickening of the glomerular basement membrane has been considered an essential pathophysiological event in diabetes-associated kidney injury. In conclusion, the results from this study show that diosgenin may protect the kidney structure and pathological changes due to diabetes mellitus.

Accumulating proof suggests that diosgenin has the ability to modulate multiple molecular targets, particularly oxidative stress and inflammation. Hyperglycemia induced oxidative stress appears to be one of the major contributing factors in the development of diabetic renal disfunction. The present study investigated the beneficial result of diosgenin on diabetes iatrogenic kidney injury. Effectiveness of diosgenin as an antioxidant and anti-inflammatory agent was evident from its effect on the renal antioxidant system and oxidative markers lipid peroxidation. Thus, diosgenin exhibited a protecting result on kidney in diabetic rats, implying that it might be a possible candidate for the treatment of diabetes and related complications. Further, pharmacological and biochemical investigations are warranted to elucidate the exact mechanism of action(s).

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