Beneficial role of magnesium valproate in diabetic nephropathy associated with renal fibrosis in experimental animals

Samir Rabadiya\textsuperscript{a}. Ashvin Dudhrejiya\textsuperscript{b}

\textsuperscript{a}. Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India
\textsuperscript{b}. B. K. Mody Government Pharmacy College, Rajkot, Gujarat, India

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Abstract

Introduction: Valproic acid is widely used as an anti-epileptic globally. Valproic acid has been shown to inhibit inflammatory cytokines, prevents fibrosis and reduce oxidative stress in various experimental models. Further, it improves glycemic control in diabetics. So, we have studied the effect of 12 week treatment with Magnesium Valproate (MgV, 210mg/kg/day, PO) on renal complications associated with streptozotocin (STZ) induced type I and type II diabetic rats.

Method: Wistar rats were made type I diabetic with STZ (35 mg/kg, IV) and type II diabetes was induced by STZ + Nicotinamide (50 mg/kg, IP + 90 mg/kg, IP). The animals were divided in groups and treatment was given for 12 weeks after which various biochemical, urine and renal antioxidants, pro-oxidant and histological parameters were estimated.

Result: STZ significantly increased blood glucose level, C-reactive protein, blood urea nitrogen, serum creatinine, serum uric acid and significantly reduced serum albumin level. Further, diabetic control animals significantly increased urine output and urinary albumin and reduced urinary creatinine and creatinine clearance. Diabetic animals showed significantly reduced level of superoxide dismutase (SOD), nitrite, reduced glutathione and catalase while increased prooxidant level of malondialdehyde and kidney collagen level. Moreover, inflammatory markers like IL-1\textbeta, IL-6 and TNF-\alpha levels were increased significantly in untreated diabetic animals. Chronic treatment with MgV significantly reduced blood glucose level, C-reactive protein, blood urea nitrogen, serum creatinine, serum uric acid and increased albumin level. MgV also significantly reduced urine output and urinary albumin and increased creatinine clearance. MgV significantly increased antioxidant enzymes and reduced collagen in kidney. Further, MgV treatment significantly reduced all inflammatory cytokines and increased protective cytokine level like IL-10. MgV reduced kidney fibrosis as evident by Masson's trichrome stain of kidney tissue.

Conclusion: Our data suggests that Magnesium Valproate prevents not only the STZ-induced nephrotic abnormalities but also renal complications which is possibly mediated through inhibition of inflammatory cytokines and reduction in blood glucose in diabetic animals.

Keywords: Diabetes, Histone deacetylase, Magnesium valproate, Nephropathy, Renal fibrosis.

1. Introduction

More than 180 million people around the world is affected by diabetes, and the number of patients is about to increase to 300 million by 2025 [1] and subsequently uncontrolled diabetes mellitus on long term produces several complications like diabetic cardiomyopathy, nephropathy, neuropathy and retinopathy. Diabetic nephropathy (DN) is a leading cause of kidney disease and end stage renal failure which affects 40-43% patient with Type 1 diabetes mellitus [2]. Without specific interventions, approximately 80% of subjects with type 1 diabetes who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of nearly 10-20% per year to the stage of overt nephropathy. End stage renal
disease develops in 50% of diabetic individuals with overt nephropathy within 10 years and in >75% by 20 years [3]. Diabetic nephropathy is characterized by the abnormal kidney function with significant glomerular and tubulointerstitial injury and fibrosis which finally leads to kidney failure [4].

The renal complications of diabetes mellitus are mediated through several intracellular and sub cellular enzymes, kinases and receptors like Estrogen receptors (ER), Histone deacetylases (HDAC), Interleukins (IL) and Tumor necrosis factor-alpha (TNF-α). The Diabetic Control and Complication Trial clearly shown that improved glycaemic control reduces risk of renal complications [5]. Despite this, it is reported that in addition to glucose control, more intensive therapy, with stringent target is required for preventing diabetic complications [6]. Hence, targeting some of above mentioned enzymes, kinases or receptor simultaneously might be an important strategy to prevent renal complications of diabetic mellitus. Oxidative stress is the initial part of DN and activates a variety of pathological pathways in virtually all types of kidney cells. However, inflammation appears to be the central role in the onset and progression of kidney fibrosis and fibrosis is the most prominent feature of DN and if uncontrolled [7].

Plasma concentrations of inflammatory molecules, including pro-inflammatory cytokines, are elevated in diabetic patients. Concentrations of these substances increase as nephropathy progresses presenting a direct association with clinical markers of glomerular and tubulointerstitial damage. The extent of inflammatory cell accumulation in the kidney is closely associated with DN. Indeed, inhibition of inflammatory markers has been shown to be protective in experimental diabetic nephropathy [7].

HDACs are a family of enzymes that remove acetyl groups from a ε-N-acetyl lysine indicating that HDAC activity is also associated with the development and progression of some chronic diseases characterized by fibrosis, including chronic kidney disease [8]. It has been reported that Trichostatin (TSA), a histone deacetylase inhibitor, significantly reduces the fibrosis in renal tubular cells and also reduces the epenchymal to mesenchymal transition (EMT). Similar results were obtained in human renal tubular cells [9, 10]. Valproic acid (VLA) in addition to its antiepileptic activity, it is also reported to have antioxidant activity [11, 12]. VLA also inhibits pro-inflammatory cytokines like IL-1β, IL-6 and TNF-α [13, 14].

VLA is also a well known inhibitor of HDAC and it selectively inhibits the catalytic activity of class I HDACs and induces proteasomal degradation of HDAC2 [15]. VLA also reduced protein expression of TGF-β and extracellular matrix (ECM) protein components in rat kidney tubular epithelial cells and attenuated epenchymal to mesenchymal transition [16].

Magnesium valproate is newly introduced salt of valproic acid and not much information is available other than its use in epilepsy. Magnesium valproate is hydrolyzed to valproic acid and magnesium ions upon absorption in blood stream and it exhibits a slower and more regular absorption rate, which prevents the variations in plasma levels of valproate typically observed when sodium salts of valproic acid are administered [17].

Although, several reports of HDACs in renal diseases are available, the role of magnesium valproate in diabetes induced renal complications and nephrotic markers have not been reported. Hence, the objective of present investigation was to study the effect of magnesium valproate on renal complications associated with type-1 diabetes in rats.

2. Materials and methods

2.1 Protocol

The protocol of the experiment was approved by institutional animal ethics committee as per the guidance of committee for the purpose of control and Supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. (IAEC/SUDPS/1310)

2.2 Study design

Healthy adult Wistar rats of either sex and 6-8 weeks of age were chosen for the study and maintained under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and 12h/12h light-dark cycle. Standard laboratory rat chew and UV-filtered water was provided ad libitum. The rats were injected intravenously (IV) with 35 mg/kg STZ
(Sigma Ltd., USA) dissolved in citrate buffer (0.1M, pH 4.5) for type I diabetes and 50 mg/kg STZ IP + 90 mg/kg Nicotinamide IP for type II diabetes. Two days after the injection of STZ, animals were checked for urine glucose levels with the help of available diagnostic kit- Diastix (Bayer Healthcare, India). The animals showing urine glucose levels >250 mg/dl were considered as diabetic.

The rats were then randomly divided into groups as follows:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Treatment and Dose</th>
<th>No. of Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-I (CON)</td>
<td>Treatment with vehicle</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Group-II (COT)</td>
<td>MgV</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Group-III (D1C)</td>
<td>STZ (35 mg/kg, IV)</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Group-IV (D2C)</td>
<td>STZ (50 mg/kg, IP) + Nicotinamide (90 mg/kg, IP)</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Group-V (D1T)</td>
<td>STZ + MgV (210 mg/kg, PO)</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Group-VI (D2T)</td>
<td>STZ + Nicotinamide + MgV (210 mg/kg, PO)</td>
<td>8</td>
</tr>
</tbody>
</table>

Magnesium Valproate was dissolved in distilled water and was administered orally at a dose of 210 mg/kg/day for 90 days in control treated and disease treated groups. Animals were maintained with free access to conventional dietary feed and water ad libitum throughout the experimental period. All animals were monitored regularly for changes in body weight and mortality throughout the course of study.

2.3. Blood sample and tissue collection and serum analysis

Blood samples were collected in clean dry centrifuge tubes as the end of experimental period from the retro orbital plexuses under light ether anaesthesia and were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 5000 rpm for 20 min and stored at -20°C until the analysis was carried out. Serum samples were analyzed for glucose, C-Reactive Protein (CRP), creatinine, blood urea, albumin and uric acid spectrophotometrically (Shimadzu UV-1601, Japan) using available biochemical diagnostic kits (Labcare Diagnostics Pvt. Ltd., India). For the measurement of urine parameters, animals were placed in metabolic cages with free access to water and food and urine was collected for 24 hours in a clean, dry beaker and filtered to remove turbidity. Urine samples were analysed for creatinine and albumin spectrophotometrically (Shimadzu UV-1601, Japan) using biochemical diagnostic kits (Labcare Diagnostics Pvt. Ltd., India). After withdrawal of blood samples from retro-orbital plexus, animals were sacrificed, kidneys were excised, extraneous tissues were separated and stored.

2.4. Measurement of molecular parameters

All the ELISA tests of IL-1β, IL-6, IL-10 and TNF-α were carried out according to manufacturer's instruction (Krishgen Biosystem, USA).

2.5. Measurement of Oxidative stress parameters

Renal tissues were minced and homogenized in phosphate buffer saline (pH 7.4) and used for estimation of oxidative stress markers. The level of superoxide dismutase (SOD) was measured by method described by Misra and Fridovich [18]. The level of reduced glutathione (GSH) was measured using method described by Moron et al. [19]. The total protein in kidney was calculated according to method described by Lowry et al. [20]. The nitrite level was estimated using method described by Dohare et al. [21] and Malondialdehyde (MDA) level was measured by method as mentioned by Ohkawa et al. [22]. Renal collagen was estimated by method as described by Drobnik et al. [23].
2.6. Fixation and processing of tissues for histopathology

A portion of the kidney was cut into 2-μm-thick sections, fixed and embedded in paraffin for histology. Paraffin embedded sections were cut and stained with Masson’s Trichome stain. The sections were observed under an photomicroscope (40× magnification) (Leica System, Germany) for fibrosis of kidney.

2.7. Statistical analysis

All the values are expressed as mean ± S.E.M. Statistical differences between groups were applied using SPSS software version 17.0 (USA). Data were considered to be statistically significant at p value < 0.05.

3. Results

3.1. Food intake and Water intake

At the end of twelve weeks treatment, STZ produced a significant (p < 0.05) increase in food and water intake in diabetic rats as compared to control rats. Treatment with MgV (210 mg/kg/day, PO) showed significant decreased intake of food and water (Fig. 1).

3.2. Biochemical parameters

As shown in Table 1, diabetic control rats exhibited a significantly (p < 0.05) increased level of serum glucose, blood urea nitrogen (BUN), serum creatinine, uric acid and significantly low level of serum albumin. Treatment with MgV (210 mg/kg/day, PO) showed a significant (p < 0.05) reduction in serum glucose, BUN, serum creatinine, uric acid and increased serum albumin in treated animals (Table 1).

3.3. Urinary parameters

As shown in Table 2, diabetic control rats exhibited a significantly (p < 0.05) increased urine output and urinary albumin and significantly lowered urinary creatinine. Treatment with MgV (210 mg/kg/day, PO) showed a significant (p < 0.05) decrease in urine output and urinary albumin and significantly increased urinary creatinine excretion (Table 2).

3.4. Molecular markers

Diabetic control rats showed significantly (p < 0.05) increased serum IL-1β, IL-6 and TNF-α levels and reduced level of IL-10 which are markers of diabetic nephropathy. Treatment with MgV (210 mg/kg/day, PO) showed a significant (p < 0.05) decrease in serum levels of IL-1β, IL-6 and TNF-α and increased level of IL-10 in treated animals (Fig. 2).

3.5. Antioxidant parameters

As shown in Table 3, diabetic control rats showed significantly (p < 0.05) decreased levels of SOD, Nitrite, Reduced glutathione, Catalase and increased level of Malondialdehyde. Treatment with MgV (210 mg/kg/day, PO) showed a significant (p < 0.05) increase in all antioxidant enzymes and significantly reduced Malondialdehyde level in treated animals (Table 3).

3.6. Renal collagen level

Diabetic rats showed significantly high level of collagen deposition in kidney. Treatment with MgV (210 mg/kg/day, PO) showed a significant reduction in renal collagen deposition (Figure 3).

3.7. Histopathology - Masson's Trichome Stain

Kidney of diabetic rats showed significant deposition of extracellular matrix. Treatment with MgV (210 mg/kg/day, PO) showed reduction in renal fibrosis as compared to untreated animals (Figure 4).
4. Discussion

In the present study, Streptozotocin (STZ) produced cardinal signs of type 1 diabetes which includes loss of body weight, polyphagia, polydyspia and polyuria which were reversed by chronic treatment with magnesium valproate. Type 1 diabetes mellitus in humans is characterized by a specific destruction of the pancreatic cells, commonly associated with immune mediated damage. STZ, a synthetic nitrosoureidoglucopyranose, that has been shown to induce multiple DNA strand breaks [24] and interfere with glucose transport [25].

The nitrosoareo moiety of STZ is responsible for its cellular toxicity, which is probably mediated through a decrease in NAD levels and the production of intracellular free radicals. The deoxyglucose moiety of STZ facilitates its transport across the cell membrane, in which the GLUT-2 glucosetransporter appears to play an essential role. The insulin-producing beta-cells of the islets of Langerhans not only express high levels of GLUT-2 transporters but also have a relatively low NAD content, making them particularly vulnerable to STZ toxicity [26, 27].

In present investigation, STZ produced severe hyperglycaemia and treatment with Magnesium valproate significantly reduced the serum glucose levels in type I and II diabetic rats. It has been reported that in diabetes, inflammatory and immunocompetent cells enter the islet and produce proinflammatory cytokines such as IL-1β, IL-12, TNF-α and IFN-γ each contribute to beta-cell destruction [28] and cell death [29, 30]. In addition to this, studies suggest that acetylation favours insulin expression, and that HDAC activity accordingly decreases insulin expression [31]. VLA is reported to be HDAC inhibitor and hence decrease in blood glucose levels are justified. Further, VLA also reported to inhibit inflammatory cytokines which prevents beta-cell destruction [13, 14]. A stimulatory effect of VLA on insulin release has been reported in human pancreatic islets [32]. Clinical studies also showed that VLA treated patients showed increased postprandial C-peptide and proinsulin levels [33].

C-reactive protein (CRP) is a type I acute phase response protein the synthesis of which is regulated by the pro-inflammatory cytokines IL-6, IL- and is a marker of systemic inflammation [34, 35]. Elevated plasma levels of CRP have been reported to be markers for endothelial cell dysfunction type 1 diabetes mellitus [36] and in children with T1DM [37]. In present study, there was a significant increase in CRP level in STZ diabetic rats and chronic treatment with Magnesium valproate significantly reduced CRP level diabetic rats. The significant reduction in CRP level in treated rats may be attributed to the ability of VLA to block inflammatory cytokines like IL-6 [14, 28].

The earliest stage in the development of diabetic renal disease is characterized by the microalbuminuria, which is usually present when nephropathy is diagnosed [38]. A substantial body of clinical and epidemiological evidence has shown that elevated serum uric acid is highly predictive of mortality in patients. According to a recent study, high levels of serum uric acid are associated with the early progressive loss of renal function in patients with diabetes [39]. In present investigation, we found significantly higher amount of uric acid in serum in STZ treated rats and treatment with Magnesium valproate significantly lowered the serum uric acid in diabetic rats.

It has been shown that, elevated level of uric acid can lead to arteriolopathy of preglomerular vessels, impaired autoregulation, glomerular hypertension, as well as endothelial dysfunction and kidney damage in hyperuricemic rats and this involves the renin-angiotensin system [40]. Magnesium valproate showed to reduce hyperuricemia and so it may prevent kidney damage associated with higher uric acid levels. Long term hyperglycaemia causes increased muscle damage and release of creatinine in to blood. Also, altered kidney function impairs creatinine excretion in nephropathy [41]. Decrease in glomerular filtration rate also affects creatinine clearance. In present study, we found significant increase in serum creatinine level decreased creatinine clearance and treatment with Magnesium valproate significantly reduced serum creatinine and increased creatinine clearance and this effect might be due to reduction in serum glucose level and reduction in inflammatory cytokines as mentioned earlier in present study.

Urea is the one of the waste product excreted by the kidney and main end product of protein metabolism. An elevation of blood urea usually signifies decreased renal function. Due to continuous catabolism of amino acids high urea will be formed from urea cycle [42]. In present study, there was a significant increase in blood urea nitrogen which is a waste product of blood urea. Chronic treatment with Magnesium valproate significantly reduced blood urea nitrogen. Diabetes is
usually associated with high catabolism of amino acid due to disturbed metabolic function in body. In present study, we also found significant reduction in serum glucose level and inflammatory cytokines and Magnesium valproate treatment thereby showed regression in diabetic state and that could be the reason behind reduction in blood urea nitrogen in treated rats.

Extensive studies demonstrated that diabetic patients with microalbuminuria have increased risk of progression to overt proteinuria, and after some time, renal failure. The progression of diabetic nephropathy from the appearance of clinical proteinuria to end stage renal failure is usually irreversible. Microalbuminuria is an important clinical finding because it is not only associated with an increased risk of progression to overt proteinuria (macroalbuminuria) and renal failure [42, 43]. Evidence suggests that nephrin located in glomerular podocyte has key role in developing proteinuria. In our findings, we found significant albuminuria and proteinuria in STZ treated rats and treatment with Magnesium valproate significantly reversed all these conditions and thus Magnesium valproate may halt progression of microalbuminuria to proteinuria and finally kidney failure. Renal function analysis in the Diabetes Control and Complications Trial (DCCT) also showed a significant association between good glycemic control and baseline creatinine clearance and between good glycemic control and risk reduction for development of microalbuminuria. The baseline findings from our study are in agreement with the DCCT. Further, hyperglycaemia and pro-inflammatory cytokines may be a factor for occurrence of albuminuria and our treatment ameliorated both thus improved albuminuria status [44].

It has been noted that IL-1β and TNF-α is a mediator of tubulointerstitial fibrosis and a culprit for renal fibrosis in diabetic state [45, 46]. Further, excessive accumulation of ECM in the kidneys and epithelial-to-mesenchymal transition (EMT) of renal tubular epithelial cells contributes to the renal fibrosis that is associated with diabetic nephropathy [9, 16]. The Histopathological indicator of fibrosis that is masson's trichome stain employed in current investigation revealed increased glomerular lesions, mesangial expansion and accumulation of extracellular matrix in diabetic rats. Treatment with Magnesium valproate reduced all this pathological changes in treated rats. HDAC1 and HDAC2 mediates proliferation of renal interstitial fibroblasts and expression of cell cycle proteins leading to increase in collagen content and finally fibrosis [47]. VLA is histone deacetylase inhibitor and inhibits HDAC1 and HDAC2 [15] and thus reduces proliferation of renal fibroblast and might have attenuated collagen deposition in kidney. Increases in intracellular glucose concentrations also yields enhanced collagen production and fibrosis. As discussed earlier, VLA reduced blood glucose level significantly and also inhibited interleukins which might be responsible for reduction in renal fibrosis in treatment group. Our findings are consistent with previous findings [48, 49] where HDAC inhibitors and interleukin antagonists reduces renal injury and fibrosis. TGF-β considered the central factor mediating glomerular hypertrophy, matrix expansion and glomerulosclerosis [47]. VLA, a histone deacetylase inhibitor is reported to inhibit glomerular and tubular injury by inhibition of TGF-β in diabetic state [9, 16]. Thus, Magnesium valproates prevents renal tissue alterations and thereby preserves kidney function.

Increased oxidative damage and prooxidant as well as deficits in antioxidants defence systems could be related to the complications in diabetes patients. During long-standing diabetes, the physiological response to combat oxidative stress is overwhelmed, resulting in an imbalance between prooxidative and antioxidative compounds which may lead to several renal complications including renal fibrosis [50]. In our study, we found low level of antioxidant enzymes in diabetic animals. Treatment with Magnesium valproate elevated antioxidant enzyme levels and attenuated fibrosis process as well as other renal injury. Valproic acid is known to possess antioxidant activity according to various studies [11, 12, 51]. Various studies suggested that antioxidant treatment prevents renal injury and attenuates further renal complications [52, 53]. Our findings are consistent with above findings where antioxidants reduced renal injury. So, apart from cytokine inhibitory activity, valproate also have antioxidant activity and helped in reducing renal complications.

5. Conclusion

In conclusion, our data suggests that Magnesium valproate prevents STZ induced metabolic abnormalities. It prevents renal complications by preserving kidney function, decreasing microalbuminuria and reducing inflammatory cytokines responsible for inflammation and fibrosis.
6. Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

7. Acknowledgement

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8. Funding

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9. Reference

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<th>Parameter</th>
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<th>D1C</th>
<th>D2C</th>
<th>D1T</th>
<th>D2T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (mg/dl)</td>
<td>81.03 ± 3.29</td>
<td>84.92 ± 3.31</td>
<td>638.40 ± 7.24***</td>
<td>448.4 ± 20.71***</td>
<td>429.8 ± 13.88###</td>
<td>264.2 ± 20.04$$$</td>
</tr>
<tr>
<td>CRP</td>
<td>3.61 ± 0.12</td>
<td>3.86 ± 0.26</td>
<td>23.88 ± 0.46***</td>
<td>18.37 ± 2.08***</td>
<td>14.52 ± 0.91##</td>
<td>10.69 ± 1.33####</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (BUN) (mg/dl)</td>
<td>15.69 ± 0.34</td>
<td>15.35 ± 0.96</td>
<td>50.45 ± 1.21***</td>
<td>52.34 ± 1.11***</td>
<td>36.07 ± 0.81####</td>
<td>34.41 ± 1.62####</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.82 ± 0.03</td>
<td>0.69 ± 0.10</td>
<td>2.10 ± 0.03###</td>
<td>2.63 ± 0.15###</td>
<td>1.66 ± 0.08#</td>
<td>1.36 ± 0.11####</td>
</tr>
<tr>
<td>Serum Uric acid (mg/dl)</td>
<td>1.58 ± 0.03</td>
<td>1.63 ± 0.02</td>
<td>5.52 ± 0.09####</td>
<td>5.83 ± 0.17####</td>
<td>3.99 ± 0.10####</td>
<td>4.39 ± 0.27####</td>
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<tr>
<td>Serum Albumin (mg/dl)</td>
<td>4.40 ± 0.05</td>
<td>4.12 ± 0.11</td>
<td>2.76 ± 0.06###</td>
<td>2.77 ± 0.06###</td>
<td>3.62 ± 0.05####</td>
<td>3.56 ± 0.03####</td>
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Results are expressed as Mean ± SEM (n=6); *P<0.05 vs control group; **P<0.01 vs control; ***P<0.001 vs control; *P<0.05 vs DC-1 group; **P<0.01 vs DC-1 group; ***P<0.001 vs DC-1 group; $P<0.05 vs DC-2 group; $$P<0.01 vs DC-2 group; $$$P<0.001 vs DC-2 group.
Table 2: Effect of MgV on urinary parameters

<table>
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<tr>
<th>Parameter</th>
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<th>D1C</th>
<th>D2C</th>
<th>D1T</th>
<th>D2T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine output (mL/day)</td>
<td>12.17 ± 1.40</td>
<td>12.00 ± 1.00</td>
<td>56.50 ± 4.63***</td>
<td>37.17 ± 6.08***</td>
<td>35.83 ± 2.21**</td>
<td>20.17 ± 1.53$$</td>
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<tr>
<td>Urinary albumin (mg/24 hrs.)</td>
<td>0.27 ± 0.07</td>
<td>0.33 ± 0.06</td>
<td>3.64 ± 0.73***</td>
<td>2.58 ± 0.36**</td>
<td>1.25 ± 0.12###</td>
<td>0.71 ± 0.12$$</td>
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<tr>
<td>Urinary creatinine (mg/dl)</td>
<td>93.01 ± 4.28</td>
<td>84.34 ± 1.99</td>
<td>25.44 ± 2.80***</td>
<td>44.21 ± 3.45***</td>
<td>44.47 ± 4.89###</td>
<td>69.39 ± 2.49$$</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>0.95 ± 0.10</td>
<td>0.86 ± 0.11</td>
<td>0.37 ± 0.04**</td>
<td>0.29 ± 0.03***</td>
<td>0.79 ± 0.07###</td>
<td>0.97 ± 0.08$$</td>
</tr>
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</table>

Results are expressed as Mean ± SEM (n=6); *P<0.05 vs control group; **P<0.01 vs control; ***P<0.001 vs control; #P<0.05 vs DC-1 group; ##P<0.01 vs DC-1 group; ###P<0.001 vs DC-1 group; $P<0.05 vs DC-2 group; $$P<0.01 vs DC-2 group; $$$P<0.001 vs DC-2 group.

Table 3: Effect of MgV on antioxidant parameters

<table>
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<th>Parameter</th>
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<th>D2C</th>
<th>D1T</th>
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</thead>
<tbody>
<tr>
<td>Superoxide dismutase (SOD) (Unit/mg protein)</td>
<td>2.96 ± 0.16</td>
<td>2.84 ± 0.10</td>
<td>1.28 ± 0.09***</td>
<td>1.53 ± 0.07***</td>
<td>2.00 ± 0.10###</td>
<td>2.22 ± 0.11$$</td>
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<tr>
<td>Nitrite (µmol NO/mg protein)</td>
<td>6.61 ± 0.64</td>
<td>6.19 ± 0.45</td>
<td>10.65 ± 0.69***</td>
<td>9.75 ± 0.66**</td>
<td>8.17 ± 0.25#</td>
<td>6.23 ± 0.58$$</td>
</tr>
<tr>
<td>Reduced glutathione (µg/mg wet tissue)</td>
<td>2.47 ± 0.05</td>
<td>2.65 ± 0.09</td>
<td>0.86 ± 0.05***</td>
<td>0.82 ± 0.08**</td>
<td>1.45 ± 0.06###</td>
<td>1.95 ± 0.03$$</td>
</tr>
<tr>
<td>Catalase (Unit/mg protein)</td>
<td>45.65 ± 3.54</td>
<td>43.11 ± 3.53</td>
<td>25.54 ± 1.91**</td>
<td>26.40 ± 1.55**</td>
<td>34.95 ± 2.26</td>
<td>38.06 ± 4.81</td>
</tr>
<tr>
<td>Malondialdehyde (nmole/g wet tissue)</td>
<td>41.60 ± 0.17</td>
<td>43.09 ± 1.32</td>
<td>162.5 ± 3.24***</td>
<td>167.60 ± 3.78***</td>
<td>119.90 ± 4.64$$</td>
<td>89.58 ± 2.84$$</td>
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Results are expressed as Mean ± SEM (n=6); *P<0.05 vs control group; **P<0.01 vs control; ***P<0.001 vs control; #P<0.05 vs DC-1 group; ##P<0.01 vs DC-1 group; ###P<0.001 vs DC-1 group; $P<0.05 vs DC-2 group; $$P<0.01 vs DC-2 group; $$$P<0.001 vs DC-2 group.
Figure 1: Effect of MgV on food intake (gm/day) and water intake (ml/day). Results are expressed as Mean ± SEM (n=6).
Figure 2: Effect of MgV on serum molecular parameters

![Graph showing serum IL-1β, IL-6, IL-10, and TNF-alpha levels](image)

Results are expressed as Mean ± SEM (n=6); *P<0.05 vs control group; **P<0.01 vs control; ***P<0.001 vs control; #P<0.05 vs DC-1 group; ##P<0.01 vs DC-1 group; ###P<0.001 vs DC-1 group; $P<0.05 vs DC-2 group; $$P<0.01 vs DC-2 group; $$$P<0.001 vs DC-2 group.

Figure 3: Effect of MgV on renal collagen

![Graph showing renal collagen levels](image)
Figure 3: Effect of MgV on renal collagen. Results are expressed as Mean ± SEM (n=6); *P<0.05 vs control group; **P<0.01 vs control; ***P<0.001 vs control; ^P<0.05 vs DC-1 group; ^^P<0.01 vs DC-1 group; ^^^P<0.001 vs DC-1 group; ^P<0.05 vs DC-2 group; ^^P<0.01 vs DC-2 group; ^^^P<0.001 vs DC-2 group.

Figure 4. Effect of MgV on renal fibrosis - Masson's trichome Stain

*Corresponding Author:  
Mr. Samir O. Rabadiya  
Department of Pharmaceutical Sciences,  
Saurashtra University, Rajkot, Gujarat, India  
Email: samirrabadiya@gmail.com