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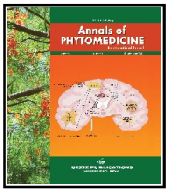

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Protective effects of diosmin in adenine-induced chronic kidney disease

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Abstract

Chronic kidney disease (Ckd) is an emerging non-communicable disease with a growing disease burden. Currently, available medications do not control its progression to end-stage renal disease. Alternate therapies such as flavonoids are researched because of their multiple activities. Diosmin, currently marketed for chronic venous insufficiency, is investigated in this study because of its reported pharmacological activities. Adenine-induced chronic kidney disease is more relevant to human Ckd than other Ckd models in rats. The molecule was investigated *in vivo* for its nephroprotective activity is proved to be effective through its antioxidant effects. At 200 mg/kg dose, diosmin has prevented the progression of Ckd by normalizing SOD, GSH, and MDA levels in rats. Also, histopathological studies reveal kidney protection in diosmin-treated groups. Thus, diosmin in the present study has proved to be protective in adenine-induced chronic kidney disease.

1. Introduction

Chronic kidney disease (Ckd), defined as the damage of the kidney or decreased kidney function for at least three months, irrespective of the cause, is characterized by reduced filtration in the glomerulus (Wilson *et al.,* 2021). The current total number of individuals affected by Ckd worldwide is estimated to be 843.6 million (Kovesdy, 2022). It is usually characterized by tubular atrophy and interstitial fibrosis. Oxidative stress, apoptosis, and fibrosis continuously occur in progressive Ckd upon exposure to nephrotoxic agents (Liyanage *et al.,* 2022). Infiltration of leucocytes, deposition of collagen, and nephritis are common histological hallmarks of kidney injury (Lv *et al.,* 2019). Uncontrolled kidney fibrosis leads to end stage renal failure with dialysis and transplantation as therapeutic options. Adenine is a nephrotoxic agent that causes tubular interstitial fibrosis, as seen in progressive Ckd (Jankowski *et al.,* 2021).

Although, risk factors like diabetes and hypertension are well controlled, the progression of Ckd to end-stage renal failure is slowly increasing, and the only therapeutic option is either dialysis or renal transplantation. Thus, complementary and alternative medicines play an important role in slowing down the progression process (Arjuna *et al.,* 2016).

Flavonoids are polyphenolics mainly used for reducing fibrosis progression in Ckd (Cao *et al.,* 2022). Diosmin is a flavone glycoside with numerous pharmacological activities, is mostly found in fruits of Citrus species. It is used for chronic venous insufficiencies and, restores blood circulation. Proven to be a potent antioxidant, it shows an inhibitory effect on TGF α in various studies (Huwait and Mobashir, 2022). It has been proven to be nephroprotective against methotrexate (Mohamed *et al.,* 2017), doxorubicin (Nemat Ali *et al.,* 2021), and cisplatin (Anwer *et al.,* 2023) in earlier studies. Adenine produces renal damage through oxidative stress and fibrosis (Awad *et al.,* 2020). In the present study, the ameliorative effects of diosmin in adenine-induced chronic kidney disease in Male Sprague Dawley (S.D.) rats were investigated.

2. Materials and Methods

2.1 Experimental animals

Male Sprague Dawley (S.D.) rats were purchased from the animal facility of Jeeva Life Sciences, Hyderabad, India. The research was conducted following CPCSEA guidelines with the previous approval of the Institutional Animal Ethics Committee (Dated 18, July 2022, Approval number CPCSEA/IAEC/JLS/18/07/22/008). The rats were kept in standard clean polypropylene cages and acclimatized for seven days in a laboratory setting with 20-22°C temp., 55 ± 5% R.H. and a 12 h light/dark cycle.

2.2 Drugs and chemicals

Adenine and diosmin were procured from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India. Losartan was obtained as a gift sample from the Care College of Pharmacy, Warangal, India. Sodium CMC and ketamine were procured from Sisco Laboratories, Hyderabad, India. A normal standard rat chow diet was purchased from Jeeva Life Sciences, Hyderabad, India. The remaining chemicals were purchased from Taranath Scientific and Chemicals, Warangal, Telangana, India.

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2.3 Experimental design

After acclimatization, rats were randomly divided into five groups (n=5):

Group I-Normal control: Vehicle (0.5 % Sodium CMC.), oral gavage

Group II-Ckd control: Adenine 200 mg/kg, oral gavage (Huang *et al.,* 2013)

Group III-Standard control: Adenine 200 mg/kg and Losartan 20 mg/kg, oral gavage

Group IV-Adenine 200 mg/kg and diosmin 100 mg/kg, oral gavage

Group V-Adenine 200 mg/kg and diosmin 200 mg/kg, oral gavage

All the experimental groups received these treatments for 28 days or four weeks continuously at the same time every day. Rats were monitored for their body weight changes and feed intake on a weekly basis. At the end of the 28th day, rats were allowed to fast overnight before the collection of blood collection. With an i.p injection of ketamine and xylazine, rats were anesthetized. The blood samples were collected from cardiac puncture. The collected blood was then made to clot for 30 min before centrifuging at 3000 rpm for 15 min. The serum was separated and well stored at – 20^{o}C until biochemical analysis. After blood collection, kidneys were immediately dissected, washed, and weighed, and a portion of tissue was fixed with 10% formaldehyde for histological studies, and the remaining tissue portion was stored in the ice-cold buffer for further analysis (Jyothilekshmi *et al.,* 2020; Venkata Lakshmi *et al.,* 2020).

2.4 Biochemical assays

The levels of creatinine, urea nitrogen, uric acid, sodium and potassium, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) were estimated in serum by standard protocols using spectrophotometry (Jyothilekshmi *et al.,* 2020).

2.5 Antioxidant parameters in kidney homogenate

Superoxide dismutase (SOD), glutathione (GSH), and lipid peroxidation marker, malondialdehyde (MDA), were estimated by methods mentioned by Patro *et al.* (2016).

2.6 Histopathology

Formalin-fixed kidney tissues were dehydrated, cleaned, and processed. 2 µm tissue sections were cut from paraffin blocks by microtome. Kidney sections were stained with H&E stain, and structural architecture was examined in 100x.

2.7 Statistical analysis

The data are expressed as mean ± SEM and were analyzed with Graph Pad Prism Version 9.5.0 for Windows software (Graph pad Software Inc., San Diego, USA). Comparisons between the groups were performed by one-way analysis of variance (ANOVA) and followed by Dunnett's Multiple Comparisons tests. p value <0.05 were considered significant (Nimisha *et al.,* 2022).

3. Results

3.1 Body weight and feed intake

The body weights of normal, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg/kg treated groups were found to be 198 ± 0.136, 138.5 ± 1.414, 177.16 ± 0.707, 184.16 ± 0.375, 179.33 ± 0.285 g, respectively. There was a significant difference between adenine and diosmin-treated groups.

The 24 h feed intake data was recorded. The average feed intake per rat in each group at the end of the fourth week was found to be 10.4 ± 0.2, 7.3 ± 1.9, 8.7 ± 1.5, 9.1 ± 1.0, 9.8 ± 0.8 g, respectively, in normal, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg and adenine + diosmin 200 mg/kg groups. There was a significant difference between adenine and diosmin-treated groups.

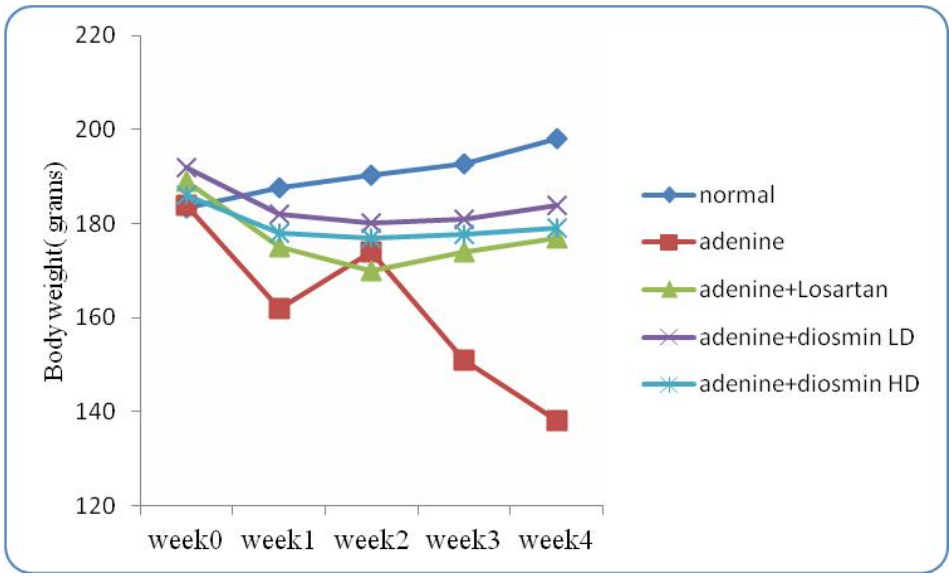


Figure 1: Effect of diosmin on body weight in adenine-induced Ckd rats.

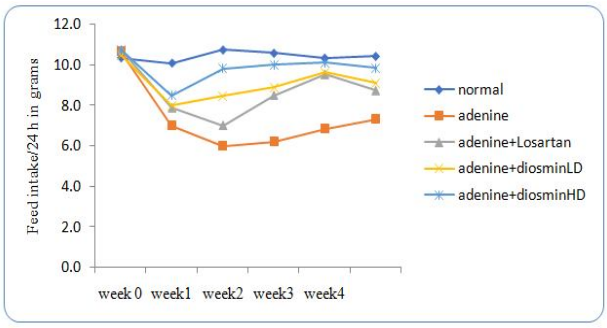


Figure 2: Effect of diosmin on 24 h feed intake in adenine-induced Ckd rats.

3.2 Kidney weight

Kidney weights of normal, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg /kg treated groups were found to be 761 ± 2.121 mg, 1162 ± 8.485, 841.8 ± 3.535, 938.2 ± 1.414 and 856.4 ± 9.192 mg, respectively. There was a significant difference between adenine and diosmin-treated groups.

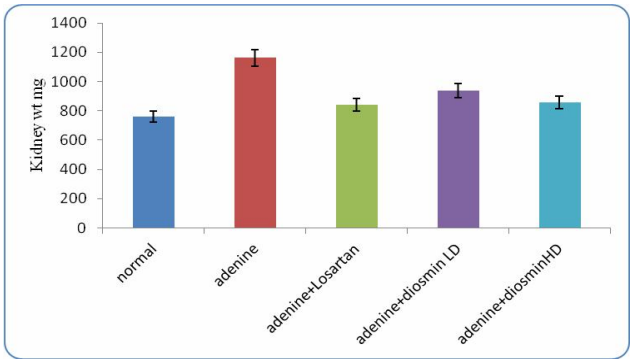


Figure 3: Effect of diosmin on kidney weights in adenine-induced Ckd rats.

3.3 Serum creatinine

Serum creatinine levels in rats treated with normal, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg/kg were found to be 0.845 ± 0.067, 4.488 ± 0.044, 2.433 ± 0.350, 3.478 ± 0.280, 2.045 ± 0.734 mg/dl, respectively. There was a significant decrease in creatinine levels in diosmin treated group compared to the adenine group.

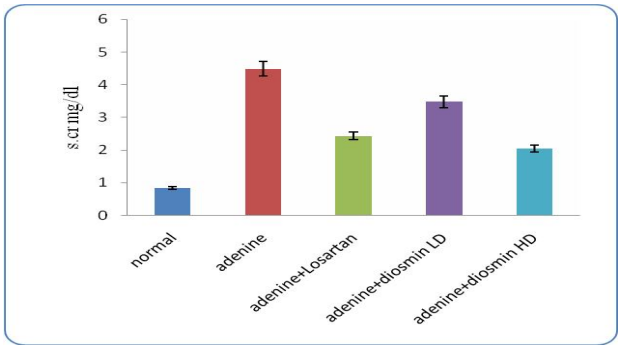


Figure 4 : Effect of diosmin on serum creatinine in adenine-induced Ckd rats.

3.4 Serum urea nitrogen

Serum urea nitrogen levels in rats treated with a normal vehicle, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg/kg were found to be 102.15 ± 17.025, 278.89 ± 4.64, 149.37 ± 2.48, 174.81 ± 2.90, 162.75 ± 2.725 mg/dl, respectively. There was a significant decrease in urea nitrogen levels in diosmin treated group compared to the adenine group.

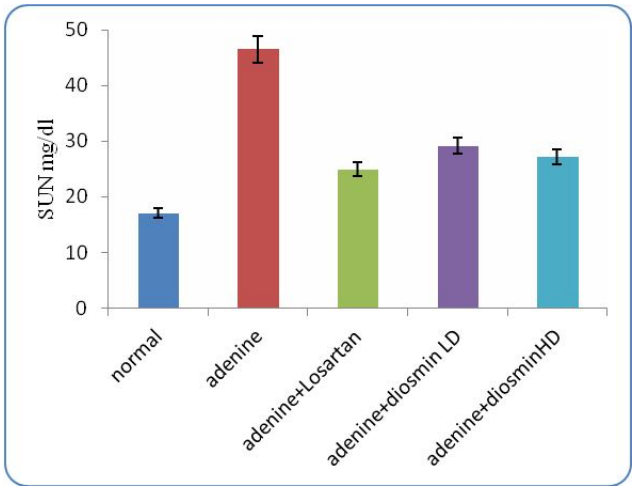


Figure 5 : Effect of diosmin on serum urea nitrogen in adenine-induced Ckd rats.

3.5 Serum uric acid

Serum uric acid levels in rats treated with a normal vehicle, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg/kg were found to be 27.03 ± 4.50, 53.32 ± 8.88, 34.05 ± 5.67, 39.66 ± 6.65, 36.41 ± 6.66 mg/dl, respectively. There was a significant decrease in uric acid levels in diosmin treated group compared to the adenine group.

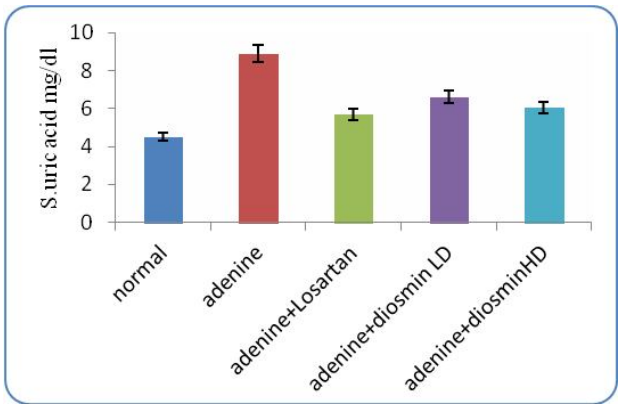


Figure 6: Effect of diosmin on serum uric acid in adenine-induced Ckd rats.

3.6 Serum electrolytes

Serum sodium levels in rats treated with normal, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg/kg were found to be 147, 133.5 ± 0.707, 143.5 ± 0.707, 139.75 ± 0.707, 140.25 ± 1.414 meq/l, respectively.

There was a significant improvement in sodium levels in diosmin treated group compared to the adenine group.

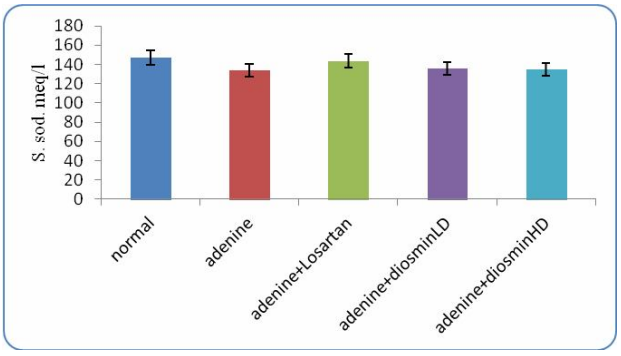


Figure 7: Effect of diosmin on serum sodium in adenine-induced Ckd rats.

Serum potassium levels in rats treated with normal, adenine 200 mg/kg, adenine + losartan 20 mg/kg, adenine + diosmin 100 mg/kg, and adenine + diosmin 200 mg/kg were found to be 4.35 ± 0353, 6.55 ± 0.070, 5.05 ± 0.070, 5.9 ± 0.141, 4.95 ± 0.070 meq/l, respectively. There was no significant decrease in potassium levels in diosmin treated group compared to the adenine group.

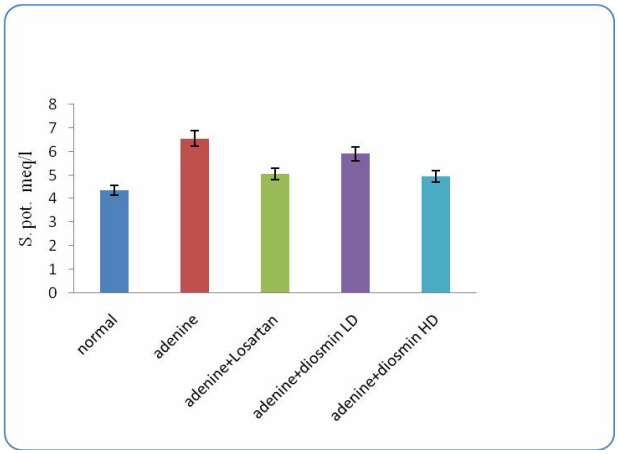


Figure 8: Effect of diosmin on serum potassium in adenine-induced Ckd rats.

3.7 Serum ALT, AST, LDH

The ALT, AST, and LDH levels were significantly raised in adenine treated group that was reduced by diosmin100 and 200 mg/kg doses. The results are tabulated below.

Table 1: Effect of diosmin on serum liver markers and LDH in adenine-induced Ckd rats

Group	ALT(IU/dl)	AST(IU/dl)	LDH(IU/dl)
Normal	26.66 ± 0.707	34 ± 0.707	135
Adenine (200 mg/kg)	59.33 ± 2.121	57.5 ± 2.121	209.33
Adenine + losartan (20 mg/kg)	32.33	37 ± 1.414	151 ± 2.82
Adenine + diosmin (100 mg/kg)	41 ± 0.707*	41 ± 0.707***	168.6 ± 3.53**
Adenine + diosmin (200 mg/kg)	34.3 ± 0.707**	36.5 ± 1.414***	150.33 ± 7.77*

Values are mean ± SEM, n = 6. All the groups were compared with the adenine control. ***p<0.001, **p<0.001, *p<0.05.

Table 2: Effect of diosmin on renal GSH, SOD, and MDA in adenine-induced Ckd rats

Group	GSH(µg/g wet tissue)	SOD(U/mg tissue)	MDA(nmol/g wet tissue)
Normal	33.50 ± 2.12	74.00 ± 2.828	27.5 ± 0.707
Adenine (200 mg/kg)	16.50 ± 0.707	39.50 ± 0.707	59 ± 1.414
Adenine + losartan (20 mg/kg)	31.00 ± 1.414	68.50 ± 0.707	29.5 ± 0.707
Adenine + diosmin (100 mg/kg)	22.00 ± 1.414*	40.50 ± 0.707	48 ± 1.414***
Adenine + diosmin (200 mg/kg)	29.50 ± 0.707***	56.00 ± 1.414***	30.5 ± 0.707***

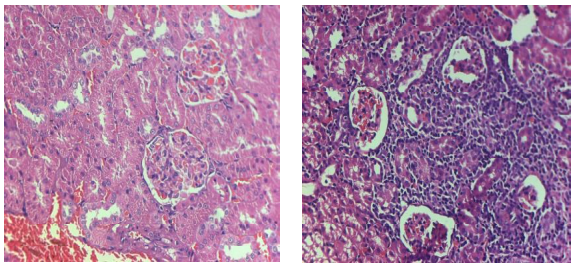
Values are mean ± SEM, n=6. All the groups were compared with the adenine control. ***p<0.001, **p<0.001, *p<0.05.

3.8 Antioxidant and lipid peroxidation marker studies in kidney

Renal antioxidant levels were significantly lowered in adenine-treated rats. Treatment with diosmin has significantly normalized the GSH and SOD levels. MDA has been significantly reduced in rats treated with diosmin 200 mg/kg than with low-dose diosmin treatment.

3.9 Histopathology

Moderate to severe infiltration of leucocytes was seen in adenine groups. Diosmin-treated groups had reduced the leukocyte infiltration in kidney sections of adenine-treated rats.



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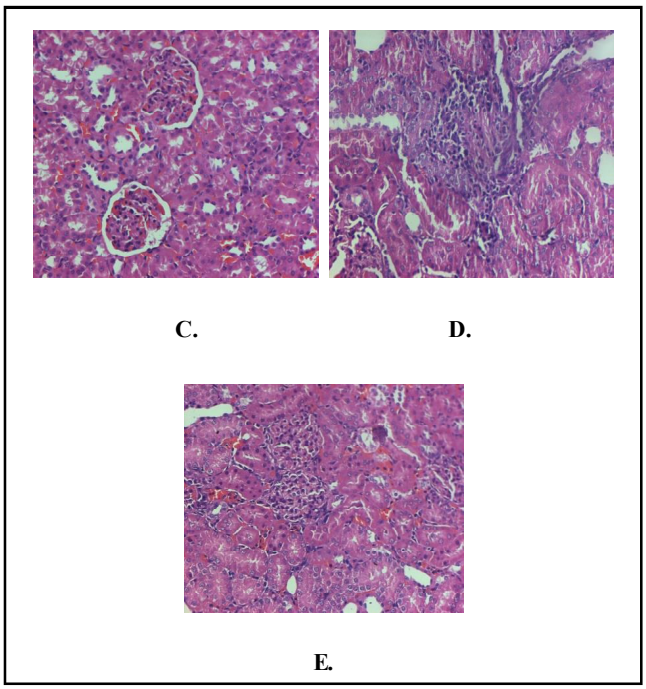

Figure 8: Histopathology of rat kidney sections.

A. Normal vehicle

B. Adenine 200 mg/kg

C. Adenine + losartan 20 mg/kg

D. Adenine + diosmin 100 mg/kg

E. Adenine + diosmin 200 mg/kg

4. Discussion

Ckd is emerging as a highly prevalent non-communicable disease characterized by slow progressive loss of nephrons. Controlling risk factors have not proved to be beneficial. This makes alternative therapies inevitable. Fibrosis is the common pathway of Ckd, and drugs focusing on reversing fibrosis are still in the research and face several challenges as the cytokines responsible for fibrosis participate in multiple signaling pathways in the body. Flavonoids are known to attenuate tissue fibrosis because of their antioxidant potential (Soheila *et al.,* 2019). Diosmin, investigated in the present study and available in the market for venous insufficiencies, is known for its antioxidant and anti-inflammatory properties (Saad Mustafa *et al.,* 2022). The study has been conducted in adenine-induced male sprague dawley rats. 100 mg and 200 mg/kg diosmin doses were selected literature of nephroprotective studies (Nemat Ali *et al.,* 2021).

From body weight and feed intake data, it was observed that adenine 200 mg/kg has significantly reduced body weights in rats and was recovered in diosmin 100 mg /kg treated groups. 200 mg/kg diosmin treated group has almost normalized the amount of feed administered by rats per week in 4 weeks in chronic kidney disease induced rats, same as with losartan 20 mg/kg treated groups. diosmin at 200 mg/kg dose significantly reduced kidney weights in adenine-treated rats.

The effect of diosmin on raised renal parameters in Ckd was studied. At 100 and 200 mg/kg doses, diosmin had reduced adenine-induced rise in serum creatinine, urea nitrogen, and uric acid. The effect was found to be significant.

Significant changes in serum electrolyte levels (sodium, potassium) were found in adenine treated group when compared to the normal group. At 200 mg/kg dose, diosmin has shown similar effects as that of losartan 20 mg/kg in adenine-treated rats and was also found to exhibit a significant reduction in ALT, AST, and LDH levels.

Adenine has reduced antioxidant enzyme superoxide dismutase, antioxidant glutathione, and increased lipid peroxidation marker, MDA, significantly in kidneys that were significantly recovered with diosmin 200 mg/kg. The effect is similar to the effect seen in losartan 20 mg/kg treated rat groups.

Histopathological studies have revealed that adenine has produced multifocal inflammation and infiltration of lymphocytes in glomerulus and tubules upon H&E staining, whereas kidney sections have mild infiltration and mild nephritis in rats treated with diosmin 200 mg/kg simultaneously with adenine. In the present study, diosmin may have shown such effects by reducing the oxidative stress in kidneys induced by adenine.

5. Conclusion

The results from the present study exhibited adenine had produced characteristic features of severe Ckd in rats, and diosmin exhibited ameliorative properties in the experimental model. The body weights, feed consumption, and kidney weights were normalized. Serum biochemical parameters were also recovered. The higher dose of diosmin, *i.e.*, 200 mg/kg, was found to be as effective as losartan 20 mg/kg in adenine-induced chronic kidney disease. This may be due to the antioxidant properties exhibited by diosmin. Further studies can be warranted on this molecule to evaluate in depth mechanism of action.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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