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Original Article

Leonurine Attenuates Streptozotocin-Induced Diabetic Nephropathy in Rats by Mitigating Dyslipidemia and Inflammation

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ABSTRACT

Background: Diabetic Nephropathy (DN) is one of the serious complications associated with a prolonged diabetic condition. DN continues to be the primary cause of morbidity and mortality among diabetic patients globally. **Objectives:** The objective of the current work is to study the nephroprotective properties of leonurine in the diabetic nephropathy model. **Materials and Methods:** The experimental rats were induced with DN through an administration of 65 mg/kg of Streptozotocin (STZ) and the DN rats were treated with leonurine for 12 weeks. Following the treatment period, the blood glucose, insulin and glycosylated Hemoglobin (HbA_{1c}) levels were evaluated. The renal function markers, including creatinine, kidney injury molecule, ROS level, lipid profiles and pro-inflammatory cytokine levels, were examined using the kits. The renal tissues of the experimental rats were subjected to the histological studies. **Results:** The outcomes of the present work found that the leonurine treatment considerably increased the insulin levels, decreased blood glucose and HbA_{1c} levels and decreased ROS production in the DN rats. In the DN rats, the treatment of leonurine significantly reduced the inflammatory markers, renal fibrotic markers, kidney dysfunction markers and lipid markers. The histological analysis results also confirmed leonurine's therapeutic effects against DN in rats. **Conclusion:** The current findings suggest that leonurine has the capacity to be a talented candidate to treat DN.

Keywords: Renal fibrosis, Hyperglycemia, Leonurine, Insulin, Dyslipidemia.

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INTRODUCTION

Diabetes Mellitus (DM) is a widely recognized endocrine complication caused by a deficiency of insulin synthesis in pancreatic tissues. The report indicates that the number of individuals affected by DM is projected to reach 300 million by 2025.[1] Diabetic Nephropathy (DN) is a chronic condition that affects diabetic patients and causes alterations in the structure and function of the kidneys, thereby leading to renal failure.[2] It is well acknowledged as a significant factor leading to glomerular dysfunction and hyperfiltration, which ultimately result in End-Stage Renal Disease (ESRD). Diabetic renal disease is characterized by specific histological and cellular characteristics, such as an expanded glomerular membrane, the extracellular matrix deposition and the degeneration of tubules.[3]

Diabetic patients have experienced various microvascular complications like nephropathy. Hypertension, obesity and prolonged high blood glucose levels are the primary causes of the onset or advancement of DN and cause dysfunction of the glomeruli and damage to the kidneys. The transition of DN to ESRD is irreparable and it is crucial to employ specific therapeutic strategies to improve renal damage.[4] DN continues to be the primary cause of morbidity as well as mortality among diabetic patients worldwide, resulting in a significant public health challenge. Chronic renal failure caused by DN is a foremost cause of the death rate of diabetic patients. Among diabetic patients, DN is the third major cause of death, following cardiovascular complications and oncological pathologies.[5] Furthermore, there is a lack of clear and conclusive therapeutic techniques for DN. The development of diabetic renal problems involves a complex association between oxidative, pro-inflammatory and fibrotic mechanisms. While the precise mechanism is not completely elucidated, it is thought that these pathways play a pivotal role in the initiation of DN.[6]

Currently, within the contemporary medical system, there is a lack of targeted therapy options for DN. The early management of this condition involves the use of antidiabetic medicines and

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angiotensin-converting enzyme inhibitors, either separately or in together, to control diabetes and inhibit the ailment from progressing to an advanced state of nephropathy.[7] Nevertheless, these strategies are less effective in inhibiting the onset of DN. During the advanced stage, patients may require dialysis or, in some cases, a kidney transplant. Conventional medications are not only expensive and have unpleasant side effects, but they may also require dosage adjustments in the event of renal failure.[8] Consequently, it is indispensable to develop potential therapeutic interventions in order to establish the successful treatment of DN without any adverse effects.

Leonurine is a well-known bioactive compound found extensively in Herba leonuri. It has gained more interest due to its several pharmacological activities. It has been well reported that leonurine has several pharmacological properties, including cardioprotective,[9] antidepressant,[10] anti-inflammatory,[11] neuroprotective[12] and antiaging[13] properties. Whereas, there are no reports on the therapeutic effects of the nephroprotective properties of leonurine on the diabetic condition. Hence, the major objective of the present work is to analyze the nephroprotective properties of leonurine in the diabetic nephropathy model.

MATERIALS AND METHODS

Experimental animals

The current study was conducted on male Wistar albino rats. The rats were caged in sterilized polypropylene enclosures in controlled laboratory settings. The temperature was set at 22-26°C, with a humidity of 40-60% and a 12 hr light/dark series. Throughout the study schedule, all rats were given unrestricted access to standard rodent food and purified drinking water. Prior to commencing the studies, all the rats were acclimatized for 7 days in laboratory settings.

Experimental design and treatment methods

Following a one-week acclimatization, all rats were divided into five groups, each containing six rats. Group I is a control and is fed only rodent food. Control rats were given a solution of buffered saline without any drugs. Group II rats administered with a single-dose of STZ (65 mg/kg). Furthermore, rats were administered a glucose (0.5%) solution to prevent mortality due to acute hypoglycemia. After three days of administering the STZ, we measured Fasting Blood Glucose (FBG) level. Rats with over 250 mg/dL of glucose were classified as diabetic and chosen for additional experiments. Group III consisted of rats that were induced with DN and treated with 30 mg/kg of leonurine for 12 weeks. Group IV rats are DN-induced and treated with 350 mg/kg of metformin for 12 weeks.

Analysis of FBG, glycated Hemoglobin (HbA_{1c}), insulin and HOMA-IR levels

The experimental rats were fasted overnight before analyzing the FBG levels. The blood from the orbital sinus was taken meticulously and analyzed for glucose levels using a glucose kit (RayBiotech, USA). The levels of HbA_{1c} in experimental rats were measured using the kit (Abcam, USA). The HbA_{1c} level was assessed by analyzing the absorbance at 450 nm. The insulin level was measured using an assay kit (Abcam, USA). The presence of insulin resistance in the experimental rats was determined by measuring the HOMA-IR level. The HOMA-IR index was determined by utilizing FBG and insulin levels in accordance with the following equation: Insulin resistance was assessed using the HOMA-IR level. The HOMA-IR level was determined with insulin and FBG levels using the equation: HOMA-IR=(FBG×Insulin level/22.5).

Analysis of lipid profiles

The lipid profile was evaluated by quantifying the amounts of total cholesterol, Triglycerides (TG), Low Density Lipoprotein (LDL), Free Fatty Acids (FFAs) and HDL using an assay kits (Abcam, USA). The tests were conducted in triplicate as per the manufacturer's specified guidelines.

Analysis kidney function markers

The kidney function of the experimental rats was examined by analyzing the creatinine, Blood Urea Nitrogen (BUN) and Lactate Dehydrogenase (LDH) levels. Spectroscopic measurement was used to determine BUN, creatinine and LDH using the respective assays using their recommended protocols (Elabscience, USA).

Analysis of KIM-1 and ROS levels

The renal tissue homogenate was utilized to analyze the levels of KIM-1 and ROS. The level of KIM-1, a well-known indicator of kidney damage, was assessed using the kits (Abcam, USA). The levels of the endogenous ROS were measured using the procedure provided by the kit's manufacturer (Cell Biolabs, USA).

Analysis of pro-inflammatory cytokines

The inflammatory cytokine levels, specifically Interleukin-1β (IL-1β), IL-6, TNF-α and TGF-β, were measured in the serum of the experimental rats using specific assay kits. The analysis was conducted following the protocols provided by the manufacturer (Abcam, USA).

Histopathological analysis

To analyze histological alterations, kidney tissues were collected and treated with 10% neutral formalin. Subsequently, the tissues were parrafinized, sectioned into 5 µm diameters and subsequently stained with Hematoxylin-Eosin (H&E). Finally,

the histopathological alterations in the kidney tissues were assessed using a microscope at a magnification of 40×.

Statistical analysis

The values were scrutinized using SPSS software and the outcomes are given as the mean±SD of three repeated experiments. The data are studied using the one-way ANOVA and Tukey's post hoc analysis, with a $p<0.05$ as significance.

RESULTS

Effect of leonurine on the FBG and HbA_{1c} level in experimental rats

Figure 1 presents the activity of leonurine treatment on the FBG and HbA_{1c} levels in the DN rats. The rats with DN exhibited remarkably heightened levels of serum FBG and HbA_{1c} when compared with control. Nevertheless, the treatment of leonurine at a 30 mg/kg dosage led to a considerable decrease in both serum FBG and HbA_{1c} levels (Figure 1). These data indicate that leonurine significantly influenced the metabolic changes in the DN rats. The metformin treatment also reduced the HbA_{1c} and FBG levels in the DN rats.

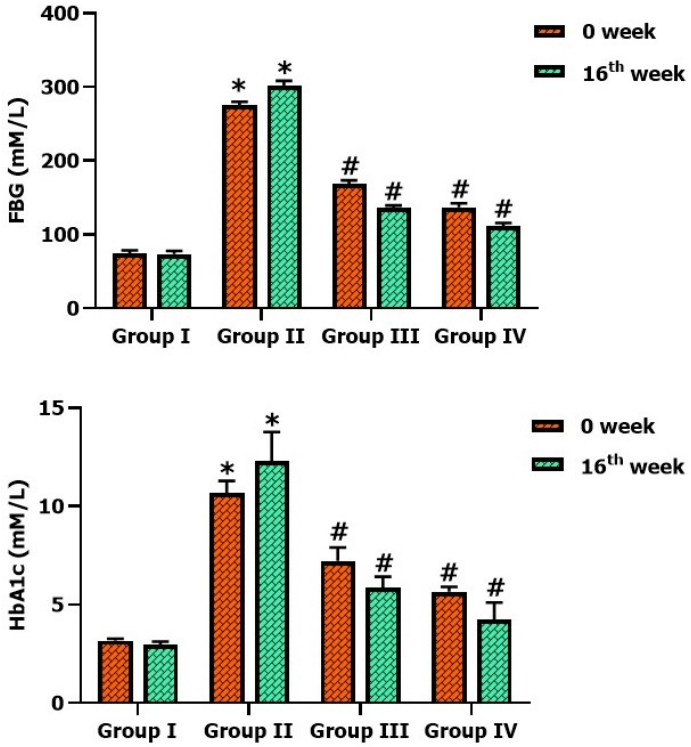

Figure 1: Effect of leonurine on the FBG and HbA_{1c} level in the experimental rats.

The data were presented as the mean±SD of three independent measurements. The statistical analysis of the results was conducted using one-way ANOVA and Tukey's post hoc test. The symbol '*' denotes statistical significance ($p<0.05$) when compared to the control (group I). The symbol '#' denotes a statistical significance ($p<0.01$) when compared to the DN-induced group (group II).

Effect of leonurine on the insulin and HOMA-IR levels in experimental rats

The insulin and HOMA-IR levels in the experimental rats were examined and the results are presented in Figure 2. The current findings exhibited that the serum insulin levels were remarkably augmented, while the HOMA-IR was considerably decreased when compared to the control. The leonurine treatment successfully boosted the insulin levels while effectively reducing the HOMA-IR status in a DN rats. This activity of leonurine was supported by the findings of the metformin treatment.

Effect of leonurine on the lipid profiles of experimental rats

Figure 3 exhibits the impact of leonurine on the lipid markers, including total cholesterol, LDL, HDL, VLDL and TG, in the experimental rats. The DN tats induced by STZ exhibited a considerable elevation in the cholesterol, LDL, VLDL and TG while reducing the HDL levels in their serum. Interestingly, the total protein, TG, LDL and VLDL levels were remarkably suppressed and HDL was successfully elevated by the leonurine in the DN rats. The lipid-lowering property of leonurine was in accordance to the findings of metformin treatment.

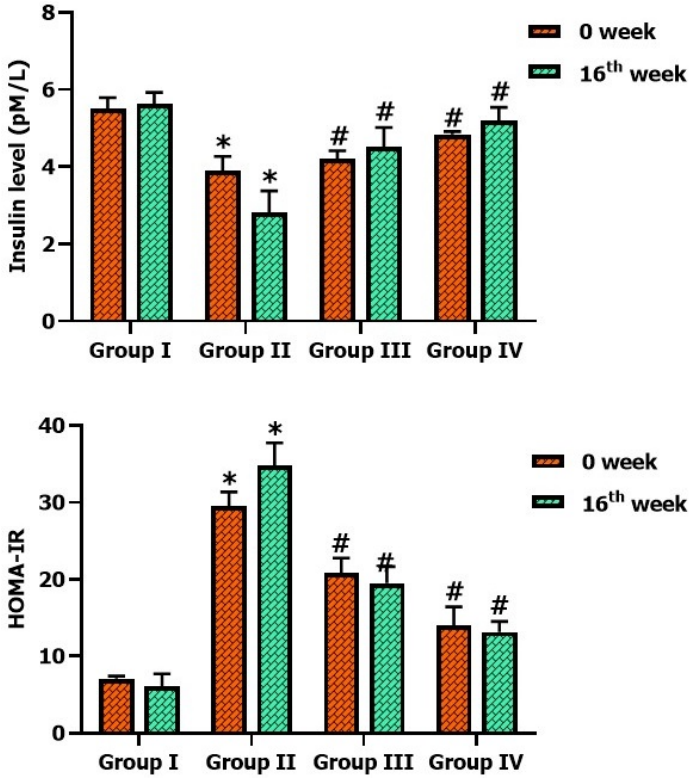

Figure 2: Effect of leonurine on the serum insulin and HOMA-IR level in the experimental rats.

The data were presented as the mean±SD of three independent measurements. The statistical analysis of the results was conducted using one-way ANOVA and Tukey's post hoc test. The symbol '*' denotes statistical significance ($p<0.05$) when compared to the control (group I). The symbol '#' denotes a statistical significance ($p<0.01$) when compared to the DN-induced group (group II).

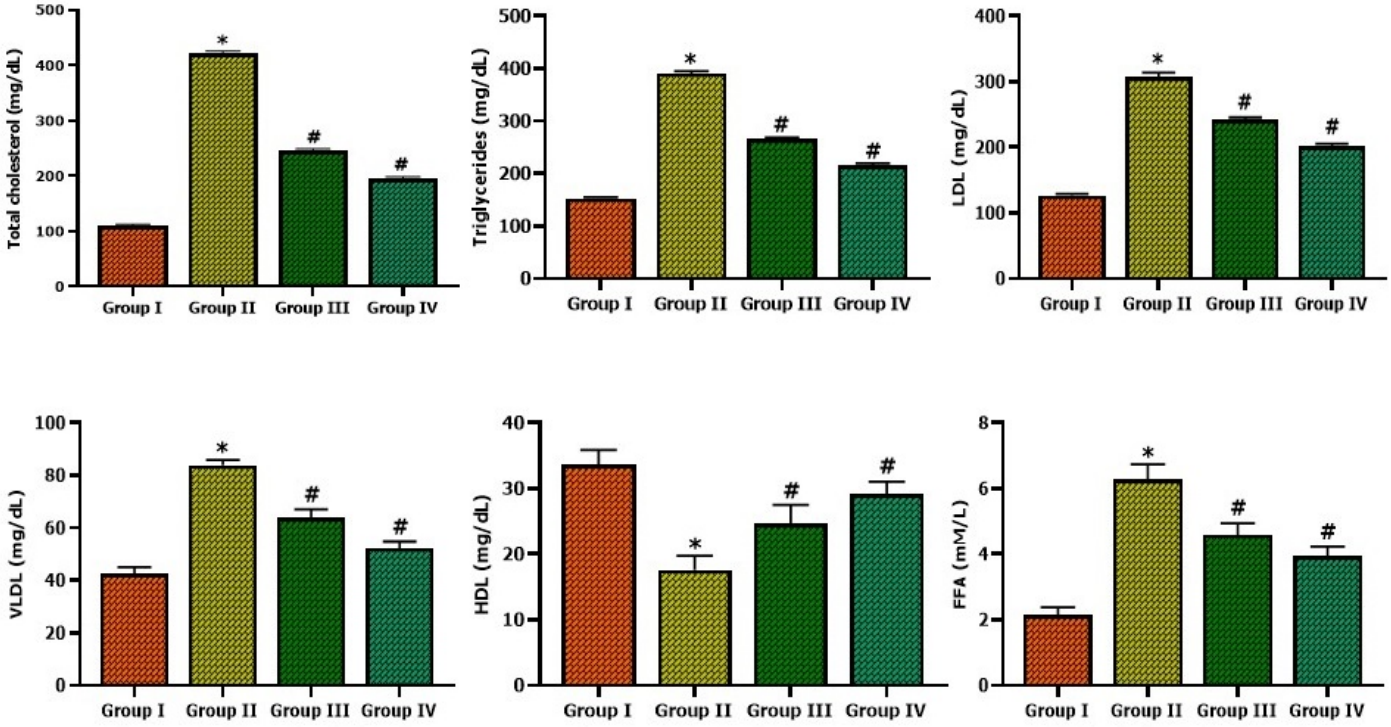

Figure 3: Effect of leonurine on the lipid profiles of the experimental rats.

The data were presented as the mean±SD of three independent measurements. The statistical analysis of the results was conducted using one-way ANOVA and Tukey's post hoc test. The symbol '*' denotes statistical significance (p<0.05) when compared to the control (group I). The symbol '#' denotes a statistical significance (p<0.01) when compared to the DN-induced group (group II).

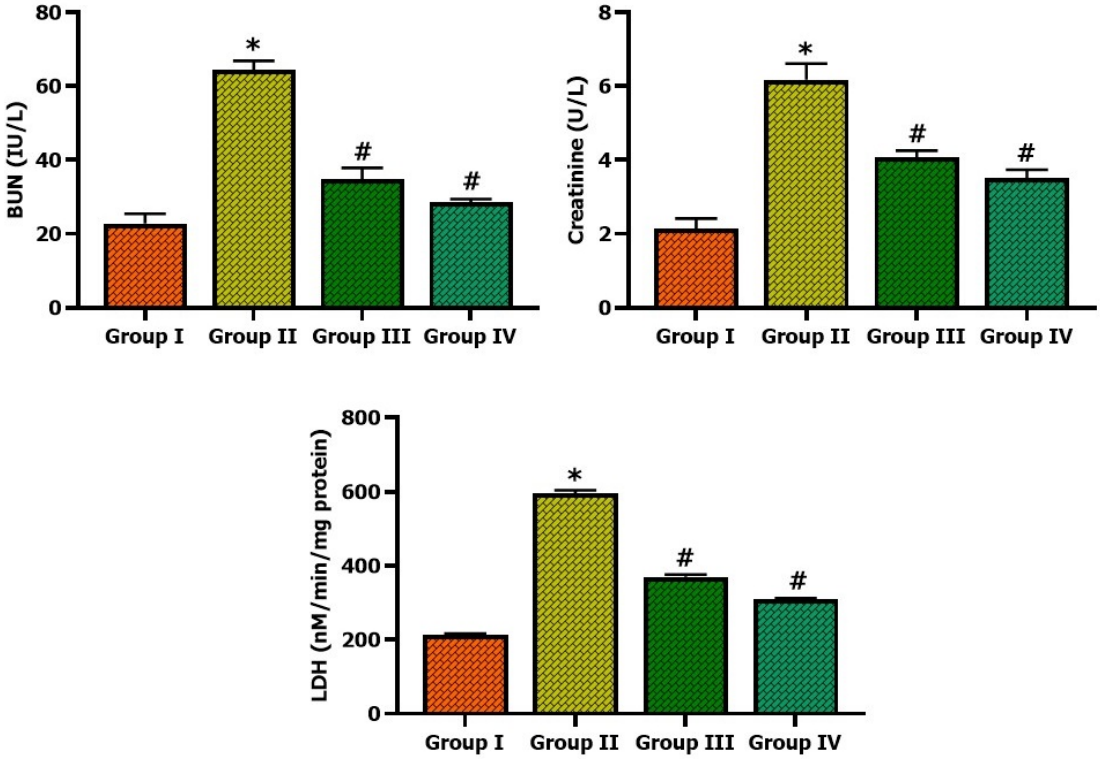

Figure 4: Effect of leonurine on the kidney function marker levels in the experimental rats.

The data were presented as the mean±SD of three independent measurements. The statistical analysis of the results was conducted using one-way ANOVA and Tukey's post hoc test. The symbol '*' denotes statistical significance (p<0.05) when compared to the control (group I). The symbol '#' denotes a statistical significance (p<0.01) when compared to the DN-induced group (group II).

Effect of leonurine on the kidney function marker levels in the experimental rats

The kidney function markers, i.e., creatinine, LDH and BUN levels, in the experimental rats were given in Figure 4. The DN rats exhibited a considerable elevation in the BUN, creatinine and LDH levels than the control. Nevertheless, the 30 mg/kg of leonurine given to DN rats led to a remarkable diminution in the BUN, creatinine and LDH levels. This clearly indicates the antioxidant capabilities of nigericin, as shown in Figure 4. These effects were also seen in the standard drug metformin-treated DN rats that highlights the leonurine's activity.

Effect of leonurine on the KIM-1 and ROS levels in experimental rats

The leonurine's effect on the KIM-1 and ROS levels in the experimental rats was assayed and the results are presented in Figure 5. The KIM-1 and ROS levels were augmented in the DN rats as compared with control rats. In contrast, the treatment with 30 mg/kg of leonurine remarkably suppressed both KIM-1 and ROS levels in the DN rats. The metformin treatment also decreased these markers in the DN.

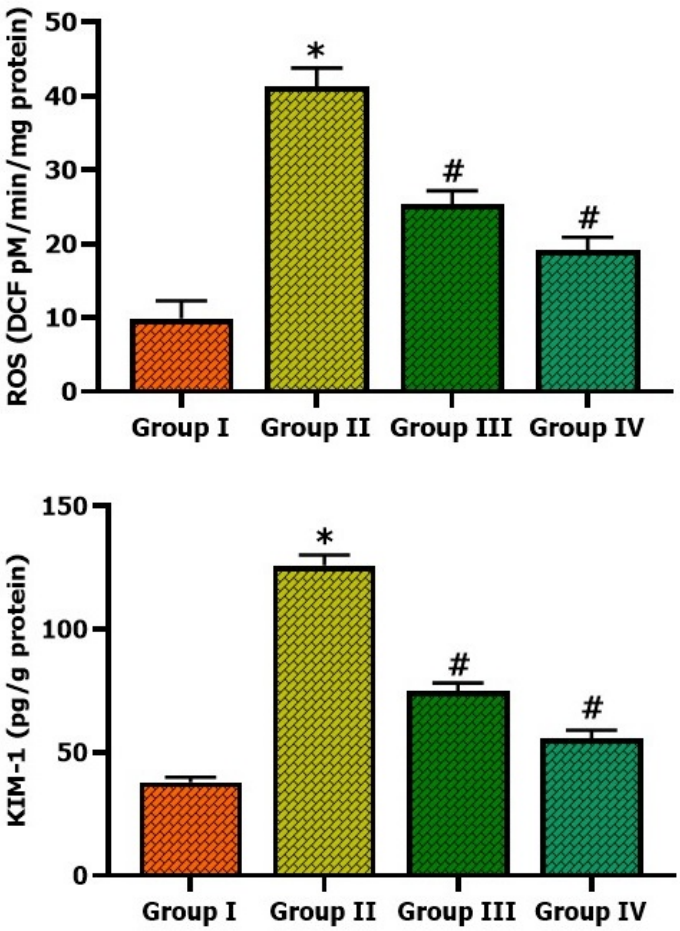

Figure 5: Effect of leonurine on the KIM-1 and ROS levels in the experimental rats.

The data were presented as the mean±SD of three independent measurements. The statistical analysis of the results was conducted using one-way ANOVA and Tukey's post hoc test. The symbol '*' denotes statistical significance ($p<0.05$) when compared to the control (group I). The symbol '#' denotes a statistical significance ($p<0.01$) when compared to the DN-induced group (group II).

Effect of leonurine on the inflammatory cytokines in experimental rats

Figure 6 demonstrates the inflammation suppressive effects of leonurine on the DN rats. The DN rats demonstrated significant elevations in the IL-6, IL-1β, TNF-α, NF-κB and TGF-β levels than the control. Remarkably, the leonurine at 30 mg/kg concentration effectively decreased these cytokines in the DN rats (Figure 6). The anti-inflammatory properties of the leonurine were in accordance to the findings of the metformin treatment, which also diminished these markers in the DN rats.

Effect of leonurine on the kidney histopathology of experimental rats

Figure 7 presents the histological observations of the kidney tissues in the experimental rats. The kidney tissues from control rats had a healthy appearance with characteristic renal histoarchitectures. Nonetheless, the renal tissues of DN rats exhibited glomerular shrinkages and necrosis, damage to renal tubular epithelial cells, degeneration, edema and an augmented infiltration of neutrophils. Remarkably, the leonurine treatment effectively mitigated these histological alterations (Figure 7). The metformin also ameliorated the STZ-induced histological changes in the kidney tissues of DN rats.

DISCUSSION

DN is a prevalent and significant microvascular complication of DM. In diabetic patients, consistently high blood glucose levels cause nephropathy, which is currently the major cause of kidney failure.[14] DN is a clinical condition characterized by reduced Glomerular Filtration Rate (GFR), enlargement of glomerular and tubular cells, excessive accumulation of ECM components and tubular and glomerular wall thickening, resulting in sclerosis and fibrosis, ultimately leading to kidney failure.[15]

The development of DN is accompanied by hemodynamic, inflammatory and metabolic changes. However, the utilization of antidiabetic drugs to regulate blood glucose levels does not effectively reduce the elevated mortality rate caused by renal and cardiovascular events linked to chronic kidney disease. It has been demonstrated that DN is an inflammatory condition characterized by inflammation and oxidative stress, leading to damage in the renal glomeruli and tubules. The presence of an excess of nitrogen, combined with a reduction in the production of proteins, initiates the formation of non-protein nitrogenous substances like BUN, creatinine and LDH in individuals with DN.[16] The elevated BUN, creatinine and LDH levels in the DN rats indicate the development of renal damage, which serves as

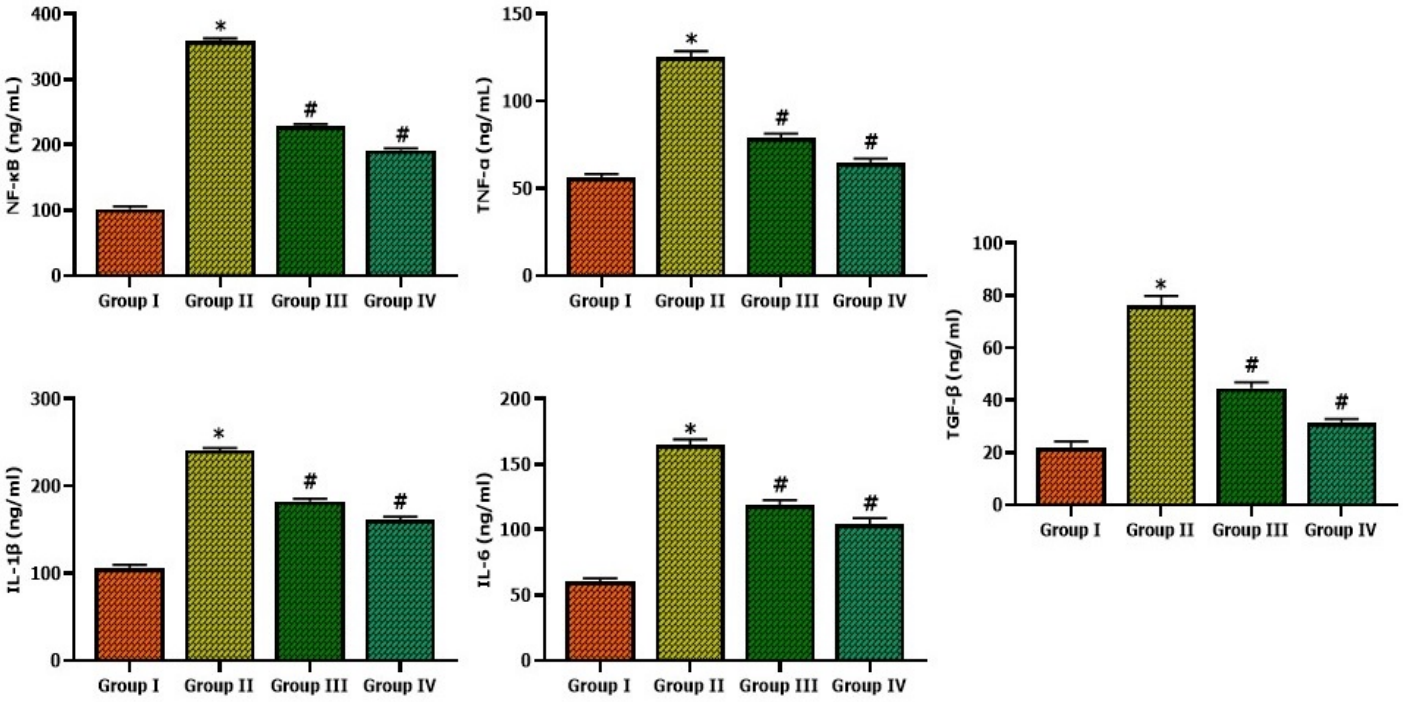

Figure 6: Effect of leonurine on the inflammatory marker levels in the experimental rats.

The data were presented as the mean±SD of three independent measurements. The statistical analysis of the results was conducted using one-way ANOVA and Tukey's post hoc test. The symbol '*' denotes statistical significance (p<0.05) when compared to the control (group I). The symbol '#' denotes a statistical significance (p<0.01) when compared to the DN-induced group (group II).

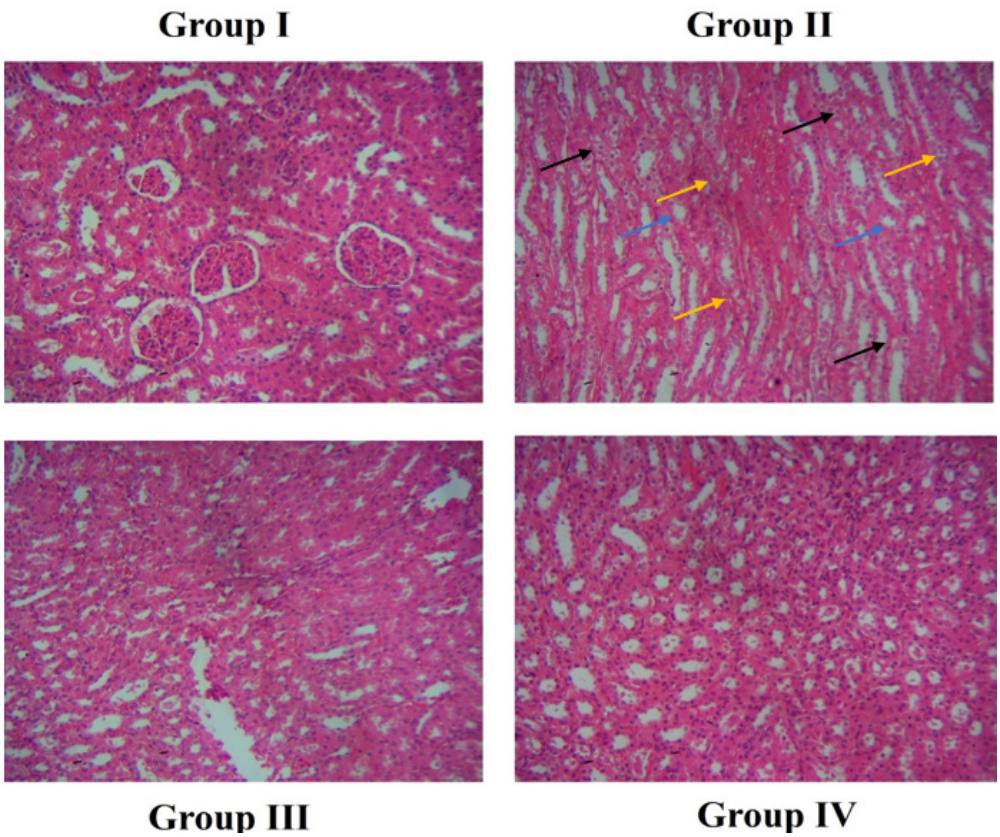

Figure 7: Effect of leonurine on the kidney histopathology of the experimental rats. Group I: Normal control; Group II: STZ-induced DN rats; Group III: DN-induced+30 mg/kg of leonurine-treated rats; Group IV: DN-induced+350 mg/kg of metformin (the standard drug)-treated rats.

Note: Yellow arrows: glomerular shrinkages and necrosis; blue arrows: tubular epithelial cell degeneration and edema; black arrows: infiltration of neutrophils.

an indicator of impaired glomerular filtration rate in DN.[17] Rats with drug-induced DN exhibited elevated levels of BUN and creatinine. The creatinine clearance is assessed by measuring the amounts of creatinine in both the blood and urine, which provides information on any alterations in renal function. In individuals with DM, it is frequently noted that there is an increase in the level of creatinine in the blood and a decrease in the urinary creatinine level, indicating impaired renal function.[18] In a similar manner, the present findings demonstrated increased BUN, creatinine and LDH levels in the DN rats. These elevations are considerably mitigated by the leonurine, which is also supported by the metformin treatment. These findings support the hypothesis that leonurine can promote kidney function in diabetic condition.

Elevated levels of HbA_{1c}, HOMA-IR and the occurrence of reduced serum insulin levels are reliable indicators for assessing the diabetic condition and DN.[19] An increase in HbA_{1c} significantly increases the likelihood of developing diabetes-related microvascular complications. Additionally, HbA_{1c} values are strongly associated with the DN.[20] The present results also found increased HbA_{1c}, HOMA-IR and reduced insulin levels in the DN rats. Remarkably, the leonurine effectively reduced the HbA_{1c} and HOMA-IR levels and augmented the serum insulin levels in the DN rats. KIM-1 is a significant marker that is produced in small amounts in the proximal tubules of healthy kidneys and is accumulated in considerable amounts in the urine during nephrotoxic damage and in response to kidney metabolic disruption. KIM-1 is the most accurate indicator of tubular damage.[21] In this study, the DN rats revealed increased KIM-1 levels when compared to the control. Whereas, the leonurine treatment remarkably attenuated the KIM-1 levels in the kidney tissues of the DN rats.

Oxidative stress occurs when there is an imbalance in the cellular redox balance due to persistent hyperglycemia and a diabetic environment, resulting in the uncontrolled generation of ROS. Prolonged hyperglycemic conditions in the advanced stages of diabetes lead to the development of advanced glycation end products. These substances have a role in the progression of DN and other problems related to the small and large blood vessels.[22] Increasing evidence supports the crucial function of oxidative stress in various clinical conditions.[23] In addition, oxidative stress is acknowledged as a pivotal component that exacerbates inflammation and fibrosis in diabetic kidneys.[24] Moreover, in diabetic patients, hyperglycemia increases ROS accumulation, resulting in long-term inflammation of the kidney and enlargement of both the glomeruli and tubules.[25] The findings of this work indicate that the DN rats showed increased ROS generation in their renal tissues. However, the leonurine treatment effectively decreased the endogenous ROS generation in the kidney tissues of the DN rats.

Renal inflammation caused by hyperglycemia is recognized as a primary component in the onset of diabetic renal damage. The diabetic renal inflammation is promoted by a series of interconnected events that involve the production of chemokines, immune cell influx and the subsequent release of pro-inflammatory cytokines.[26] Multiple reports have documented an abnormally high flow of immune cells from the bloodstream into the kidneys of diabetic animals and an increased accumulation of specific inflammatory mediators within the kidneys.[27,28] Inflammatory cytokines, specifically NF-κB, IL-6, IL-1β and TNF-α, are significant contributors to the progression of DN.[29] An excessive synthesis of these markers results in inflammatory alterations in the renal tissues. The NF-κB signaling has a major role in the progression of inflammatory disorders, including DM and its associated consequences, like DN. TNF-α induces kidney hypertrophy and hyperfiltration in the initial phase of DN.[30] IL-6 induces the accumulation of ECM in mesangial cells, leading to the growth of the mesangium and glomerular wall thickening. This finally leads to aberrant structural changes in the glomerulus. Additionally, IL-1β serves a pivotal role in the progression of DM and also participates in the DN development.[31] The results of this work showed that NF-κB, IL-6, IL-1β and TNF-α were drastically increased in the DN rats. Remarkably, the treatment with leonurine substantially decreased these marker levels in the DN rats. These findings highlighted the fact that leonurine has anti-inflammatory properties.

Renal fibrosis continues to be a difficult clinical condition of DN. TGF-β is recognized for its role in facilitating tissue repair. However, elevated glucose levels can trigger the abnormal TGF-β activation, leading to overproduction of matrix and the progression of renal fibrosis.[32] Patients and murine models with DN have shown increased levels of TGF-β and its downstream signaling parts.[33] The outcomes of the present study clearly demonstrated the leonurine treatment considerably decreased the TGF-β level in the DN rats.

Dyslipidemia is a condition that is connected with an elevated DN risk and other consequences because it can alter the cellular metabolism. DM-associated dyslipidemia is characterized by elevated cholesterol, FFAs, TGs, LDL and VLDL levels, together with a reduced HDL level.[34] Nevertheless, insufficient insulin levels trigger lipogenesis, contribute to dyslipidemia and boost the input of FFAs from the peripheral tissues. These mechanisms lead to muscle atrophy and depletion of adipose tissue, resulting in a fast and substantial decrease in overall body weight in patients with DM.[35] The link between dyslipidemia and diabetic complications is well reported.[36] Elevated levels of TGs and total cholesterol, together with reduced levels of HDL, are frequently observed in DM patients. The measurement of cholesterol, TG and HDL levels is crucial for evaluating lipid metabolism in DM patients.[37] The present work found that the DN rats showed increased cholesterol, TG, LDL and VLDL while having a reduced HDL level. Remarkably, the levels of lipid profiles were considerably decreased by the leonurine in the DN rats. These

findings highlight the antilipidemia properties of leonurine in diabetic conditions.

The various components of the nephrons of the renal tissue are damaged in DN. The membrane in glomeruli undergoes thickening, whereas the mesangium expands as a result of the excessive buildup of ECM. In the initial phases of DN, there is an enlargement of the renal tubules known as renal tubular hypertrophy. In addition, the changes in the structure of the kidney are accompanied by a loss of function.[38] The main clinical observations in individuals with DN comprise gradual glomerular hypertrophy, enlargement of mesangial cells, a decline in the GFR, damage to the tubules, interstitial fibrosis and the presence of albuminuria.[39] In the current study, the findings of the histopathological analysis proved that leonurine effectively mitigated the STZ-induced histological changes in the kidney tissues of the DN rats.

CONCLUSION

Overall, the present findings indicate that leonurine may have potential for mitigating DN in rats through its therapeutic effects. The treatment of leonurine significantly enhanced insulin levels, reduced FBG levels, decreased lipids and decreased kidney dysfunction marker levels in the DN rats. The leonurine treatment remarkably decreased the inflammation level, renal fibrosis and kidney injury in the DN rats. Ultimately, these results highlight that leomurine has the capacity to be a promising choice to treat DN.

ACKNOWLEDGEMENT

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ETHICS APPROVAL

This work was approved by the institutional ethical committee Hebei University, Baoding, 071000, China.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ESRD: End-stage renal disease; **DN:** Diabetic nephropathy; **TG:** Triglycerides, **LDL:** Low density lipoprotein, **FFA:** Free fatty acids; **BUN:** Blood urea nitrogen, **LDH:** Lactate dehydrogenase.

SUMMARY

Diabetic nephropathy continues to be the primary cause of morbidity as well as mortality among diabetic patients worldwide, resulting in a significant public health challenge. Chronic renal failure caused by Diabetic nephropathy is a foremost cause of the death rate of diabetic patients. The outcomes of the present work found that the leonurine treatment considerably increased the insulin levels, decreased blood glucose and HbA1c levels and decreased ROS production in the DN rats. In the DN rats, the treatment of leonurine significantly reduced the inflammatory markers, renal fibrotic markers, kidney dysfunction markers and lipid markers.

REFERENCES

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, *et al.* IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119. doi: 10.1016/j.diabres.2021.109119, PMID 34879977.
2. Abdou HM, Abd Elkader HA. The Potential Therapeutic Effects of *Trifolium alexandrinum* Extract, Hesperetin and quercetin against Diabetic Nephropathy via Attenuation of Oxidative Stress, Inflammation, GSK-3β and Apoptosis in Male Rats. Chem Biol Interact. 2022;352:109781. doi: 10.1016/j.cbi.2021.109781, PMID 34922902.
3. Zhang QY, Xu SJ, Qian JC, Yang LB, Chen PQ, Wang Y, *et al.* Pharmacological inhibition of MyD88 suppresses inflammation in tubular epithelial cells and prevents diabetic nephropathy in experimental mice. Acta Pharmacol Sin. 2022;43(2):354-66. doi: 10.1038/s41401-021-00766-6, PMID 34552217.
4. Keane WF, Brenner BM, De Zeeuw D, Grunfeld JP, McGill J, Mitch WE, *et al.* The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. Kidney Int. 2003;63(4):1499-507. doi: 10.1046/j.1523-1755.2003.00885.x, PMID 12631367.
5. González-Pérez A, Saez M, Vizcaya D, Lind M, Rodriguez LG. Incidence and risk factors for mortality and end-stage renal disease in people with type 2 diabetes and diabetic kidney disease: A population-based cohort study in the UK. BMJ Open Diabetes Res Care. 2021;9(1):002146. doi: 10.1136/bmjdrc-2021-002146, PMID 34607828.
6. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, *et al.* IDF diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271-81. doi: 10.1016/j.diabres.2018.02.023, PMID 29496507.
7. Foggensteiner L, Mulroy S, Firth J. Management of diabetic nephropathy. J R Soc Med. 2001;94(5):210-17. doi: 10.1177/014107680109400504, PMID 11385086.
8. Tabatabaei-Malazy O, Larijani B, Abdollahi M. Targeting metabolic disorders by natural products. J Diabetes Metab Disord. 2015;14:57. doi: 10.1186/s40200-015-0184-8, PMID 26157708.
9. Gao H, Yang XH, Gu XF, Zhu YZ. Synthesis and biological evaluation of the codrug of leonurine and Aspirin as cardioprotective agents. Bioorg Med Chem Lett. 2016;26(19):4650-54.[WU1]. doi: 10.1016/j.bmcl.2016.08.058, PMID 27575471.
10. Jia MM, Li CX, Zheng Y, Ding XJ, Chen M, Ding JH, *et al.* Leonurine exerts antidepressant-like effects in the chronic mild stress-induced depression model in mice by inhibiting neuroinflammation. Int J Neuropsychopharmacol. 2017;20(11):886-95. doi: 10.1093/ijnp/pyx062, PMID 29016795.
11. Jin M, Li Q, Gu YT, Wan B, Huang JF, Xu XB *et al.* Leonurine suppresses neuroinflammation through promoting oligodendrocyte maturation. J Cell Mol Med. 2019;23(2):1470-85. doi: 10.1111/jcmm.14053, PMID 30556290.
12. Liu HC, Zhang XJ, Du YY, Ji H, Li SY, Li LT, *et al.* Leonurine protects brain injury by increased activities of UCP4, SOD, CAT and Bcl-2, decreased levels of MDA and Bax and ameliorated ultrastructure of mitochondria in experimental stroke. Brain Res. 2012;1474:73-81. doi: 10.1016/j.brainres.2012.07.028, PMID 22842526.
13. Chen P, Chen F, Zhou BH. Leonurine ameliorates D-galactose-induced aging in mice through activation of the Nrf2 signalling pathway. Aging. 2019;11(18):7339-56. doi: 10.18632/aging.101733, PMID 31527304.
14. Donate-Correa J, Luis-Rodríguez D, Martín-Núñez E, Tagua VG, Hernández-Carballo C, Ferri C, *et al.* Inflammatory targets in diabetic nephropathy. J Clin Med. 2020;9(2):458. doi: 10.3390/jcm9020458, PMID 32046074.
15. Zhu L, Han J, Yuan R, Xue L, Pang W. Berberine ameliorates diabetic nephropathy by inhibiting TLR4/NF-κB pathway. Biol Res. 2018;51(1):9. doi: 10.1186/s40659-018-0157-8, PMID 29604956.
16. Tuttle KR, Agarwal R, Alpers CE, Bakris GL, Brosius FC, Kolkhof P, *et al.* Molecular mechanisms and therapeutic targets for diabetic kidney disease. Kidney Int. 2022;102(2):248-60. doi: 10.1016/j.kint.2022.05.012, PMID 35661785.

17. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes. 2008;57(6):1446-54. doi: 10.2337/db08-0057, PMID 18511445.
18. Nisha R. Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. J Clin Pathol Lab Med. 2017;1(2):1-5.
19. Selby NM, Taal MW. An updated overview of diabetic nephropathy: diagnosis, prognosis, treatment goals and latest guidelines. Diabetes Obes Metab. 2020; 22; Suppl 1: 3-15. doi: 10.1111/dom.14007, PMID 32267079.
20. Khan NU, Lin J, Liu X, Li H, Lu W, Zhong Z, *et al.* Insights into predicting diabetic nephropathy using urinary biomarkers. Biochim Biophys Acta Proteins Proteom. 2020;1868(10):140475. doi: 10.1016/j.bbapap.2020.140475, PMID 32574766.
21. Latoch E, Konończuk K, Muszyńska-Rosłan K, Taranta-Janusz K, Wasilewska A, Szymczak E, *et al.* Urine NGAL and KIM-1-Tubular Injury Biomarkers in Long-Term Survivors of Childhood Solid Tumors: a Cross-Sectional Study. J Clin Med. 2021;10(3):399. doi: 10.3390/jcm10030399, PMID 33494327.
22. King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol. 2004;122(4):333-8. doi: 10.1007/s00418-004-0678-9, PMID 15257460.
23. Tanase DM, Gosav EM, Anton MI, Floria M, Seritean Isac PN, Hurjui LL, *et al.* Oxidative stress and NRF2/KEAP1/ARE pathway in diabetic kidney disease (DKD): new perspectives. Biomolecules. 2022;12(9):1227. doi: 10.3390/biom12091227, PMID 36139066.
24. Maruno S, Tanaka T, Nangaku M. Exploring molecular targets in diabetic kidney disease. Kidney Res Clin Pract. 2022; 41; (Suppl. S2):S33-S45. doi: 10.23876/j.krcp.21.251, PMID 35354246.
25. Jha JC, Banal C, Chow BS, Cooper ME, Jandeleit-Dahm K. Diabetes and kidney disease: role of oxidative stress. Antioxid Redox Signal. 2016;25(12):657-84. doi: 10.1089/ars.2016.6664, PMID 26906673.
26. Sun H, Liu X, Long SR, Wang T, Ge H, Wang Y, *et al.* Antidiabetic effects of pterostilbene through PI3K/Akt signal pathway in high fat diet and STZ-induced diabetic Rats. Eur J Pharmacol. 2019;859:172526. doi: 10.1016/j.ejphar.2019.172526, PMID 31283935.
27. Patial V, Katoch S, Chhimwal J, Singh PP, Suresh PS, Padwad Y. *Tinospora cordifolia* activates PPARγ pathway and mitigates glomerular and tubular cell injury in diabetic kidney disease. Phytomedicine. 2021;91:153663. doi: 10.1016/j.phymed.2021.153663, PMID 34358759.
28. Xiao Y, Deng J, Li C, Gong X, Gui Z, Huang J, *et al.* Epiberberine ameliorated diabetic nephropathy by inactivating the angiotensinogen (Agt) to repress TGFβ/Smad2 pathway. Phytomedicine. 2021;83:153488. doi: 10.1016/j.phymed.2021.153488, PMID 33571918.
29. Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008;19(3):433-42. doi: 10.1681/ASN.2007091048, PMID 18256353.
30. Ahmed S, Mundhe N, Borgohain M, Chowdhury L, Kwatra M, Bolshette N, *et al.* Diosmin modulates the NF-kB signal transduction pathways and downregulation of various oxidative stress markers in alloxan-induced diabetic nephropathy. Inflammation. 2016;39(5):1783-97. doi: 10.1007/s10753-016-0413-4, PMID 27492452.
31. Lei Y, Devarapu SK, Motrapu M, Cohen CD, Lindenmeyer MT, Moll S, *et al.* Interleukin-1β Inhibition for Chronic Kidney Disease in Obese Mice With Type 2 Diabetes. Front Immunol. 2019;10:1223. doi: 10.3389/fimmu.2019.01223, PMID 31191559.
32. Ram C, Gairola S, Syed AM, Verma S, Mugale MN, Sahu BD. Carvacrol preserves antioxidant status and attenuates kidney fibrosis via modulation of TGF-B1/Smad signaling and inflammation. Food Funct. 2022;13(20):10587-600. doi: 10.1039/d2fo01384c, PMID 36156620.
33. Sureshbabu A, Muhsin SA, Choi ME. TGF-β signaling in the kidney: profibrotic and protective effects. Am J Physiol Ren Physiol. 2016; 310(7):F596-606. doi: 10.1152/ajprenal.00365.2015, PMID 26739888.
34. Hirano T, Satoh N, Kodera R, Hirashima T, Suzuki N, Aoki E, *et al.* Dyslipidemia in diabetic kidney disease classified by proteinuria and renal dysfunction: A cross-sectional study from a regional diabetes cohort. J Diabetes Investig. 2022;13(4):657-67. doi: 10.1111/jdi.13697, PMID 34665936.
35. Mottalib A, Kasetty M, Mar JY, Elseaidy T, Ashrafzadeh S, Hamdy O. Weight management in patients with type 1 diabetes and obesity. Curr Diab Rep. 2017;17(10):92. doi: 10.1007/s11892-017-0918-8, PMID 28836234.
36. Biddinger SB, Hernandez-Ono A, Rask-Madsen C, Haas JT, Alemán JO, Suzuki R, *et al.* Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. Cell Metab. 2008;7(2):125-34. doi: 10.1016/j.cmet.2007.11.013, PMID 18249172.
37. Yan Z, Fan R, Yin S, Zhao X, Liu J, Li L *et al.* Protective effects of *Ginkgo biloba* leaf polysaccharide on nonalcoholic fatty liver disease and its mechanisms. Int J Biol Macromol. 2015;80:573-80. doi: 10.1016/j.ijbiomac.2015.05.054, PMID 26047899.
38. Kolset SO, Reinholt FP, Jenssen T. Diabetic nephropathy and extracellular matrix. J Histochem Cytochem. 2012;60(12):976-86. doi: 10.1369/0022155412465073, PMID 23103723.
39. Zoja C, Xinaris C, Macconi D. Diabetic nephropathy: novel molecular mechanisms and therapeutic targets. Front Pharmacol. 2020;11:586892. doi: 10.3389/fphar.2020.586892, PMID 33519447.

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