ORIGINAL ARTICLE

Modulation of Inflammatory Markers by Vitamin D and Pioglitazone to Combat Renal Dysfunction in Type 2 Diabetic Rats

Snehal Joshi, Rajesh A. Maheshwari

Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India

Abstract

Aim: This study is designed to assess the outcomes of Vitamin D (Vit D) and Pioglitazone (PIO) on metabolic control, kidney function, inflammatory markers, and histopathology in rats with streptozotocin-nicotinamide-induced diabetes. **Materials and Methods:** Rats were randomly assigned to five groups: Normal control, diabetic control (DC), Vit D-treated, PIO-treated, and combined Vit D + PIO-treated, with treatments administered orally for 8 weeks. Blood samples were analyzed for glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), blood urea nitrogen (BUN), creatinine, and inflammatory markers, and kidney tissues were assessed histopathologically after euthanasia. **Results:** Both Vit D and PIO treatments reduced glucose, insulin, and HOMA-IR levels, with the combined treatment showing the most significant improvements, suggesting a synergistic effect. Both Vit D and PIO also reduced serum creatinine and BUN levels, with the combination therapy leading to a more substantial reduction in creatinine, although no significant improvement in BUN was observed compared to Vit D alone. The combination therapy significantly reduced C-reactive protein, monocyte chemoattractant protein-1, and interleukin-6 levels compared to the DC, though no additional benefits were observed for tumor necrosis factor-alpha, cystatin-C, and transforming growth factor-beta 1. Histopathological analysis revealed mild degenerative changes in the DC group, while the combined Vit D + PIO treatment restored kidney structure to near normal, with minimal vascular congestion. **Conclusion:** Overall, both Vit D and PIO positively impacted glucose regulation, kidney function, and inflammatory markers in diabetic rats, with combination therapy showing superior efficacy, particularly in improving metabolic control and kidney histology.

Key words: Creatinine, diabetes, inflammatory markers, kidney, pioglitazone, vitamin D

INTRODUCTION

Renal dysfunction is a prevalent setback of type 2 diabetes mellitus (T2DM) and significantly contributes to the advancement of chronic kidney disease.[1] Diabetic nephropathy (DN) is a chronic, progressive disorder marked by kidney inflammation, fibrosis, and reduced glomerular filtration capacity. The development of DN involves a complex interplay of various biological pathways, including the activation of pro-inflammatory factors and growth factors that contribute to kidney injury.[2] Key inflammatory markers, such as monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta 1 (TGF-β1), have been well-known as critical players in the onset and progression of DN.[3,4] Furthermore, higher levels of biomarkers such as C-reactive protein (CRP) and cystatin-C are commonly linked to kidney dysfunction and are frequently used in clinical practice to monitor renal damage.[5,6]

Vitamin D (Vit D) deficiency has been allied with a pre-eminent risk of kidney disease and various metabolic disorders, including diabetes.[7] Studies indicate that Vit D plays an essential role in controlling immune responses, alleviating inflammation, and boosting endothelial function.[8,9] Moreover, experimental evidence suggests that Vit D supplementation can slow the progression of DN by suppressing inflammatory pathways and preserving kidney

Address for correspondence:
Rajesh A. Maheshwari, Department of Pharmacy,
Sumandeep Vidyapeeth Deemed to be University,
Piparia, Vadodara - 391760, Gujarat, India.
Phone: +91-2668-245279.
E-mail: rajpharma2007@gmail.com

Received: 24-12-2024
Revised: 13-02-2025
Accepted: 08-03-2025

function.[10,11] In addition to Vit D, pioglitazone (PIO) helps reduce hepatic damage by modulating inflammation, apoptosis, and oxidative stress.[12]

This study seeks to explore the combined effects of Vit D and PIO on renal dysfunction and inflammatory pathways in a rat model of T2DM. We hypothesize that the dual treatment will mitigate diabetes-induced inflammation and kidney damage, as reflected by alterations in above-mentioned key biomarkers. The findings from this research could offer valuable insights into novel therapeutic approaches for managing DN, emphasizing the role of Vit D and PIO in regulating critical inflammatory pathways involved in renal impairment.

MATERIALS AND METHODS

Drugs and chemicals

PIO was acquired from Bharat Parenteral Limited in Vadodara, India, and Vit D was purchased from local markets. Streptozotocin (STZ) and nicotinamide (NA) were acquired from HiMedia, Mumbai, India. The diagnostic kits utilized in the research were sourced from a reliable supplier, and all other reagents and chemicals utilized were of high analytical-grade quality.

Experimental animals

The research design was permitted by the Institutional Animal Ethics Committee and adhered the standards guidelines of the Committee for Control and Supervision of Experiments on Animals. It involved healthy adult Wistar rats, ranging in weight from 200 to 250 g and of both genders. They were retained in polypropylene cages in a precise setting (12-h light/dark cycle, 24°C temperature, and 35–60% humidity) and were served a normal diet, with access to purified drinking water.

Experimental design

Diabetes was persuaded in the rats by administering a mixture of STZ and NA.[11] The rats were randomly allotted to five groups, each holding six animals. Group I acted as the normal control (NC), while Group II, the diabetic control (DC), received no treatment. Group III was treated with Vit D (400 IU/kg/day),[13] Group IV with PIO (20 mg/kg),[14] and Group V received both Vit D and PIO. All treatments were given orally once a day for 8 weeks. Upon completion of the study, blood was drawn from the retro-orbital plexus using glass capillaries under light ether anesthesia. The samples were stored either with or without disodium ethylenediaminetetraacetate for subsequent biochemical analysis. The blood was permitted to clot for 15 min, sample was centrifuged at 5000 rpm for 20 min to isolate the serum, which was subsequently stored at −20°C for future analysis. Biochemical markers such as glucose, gamma-glutamyl transferase, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), blood urea nitrogen (BUN), and creatinine were assessed using standard kits. In addition, serum levels of inflammatory markers were measured using enzyme-linked immunosorbent assay.

Histopathology

After euthanasia, kidney tissues from each group were promptly dissected, irrigated with saline, and fixed in 10% phosphate-buffered formalin. The samples were subsequently imbedded in paraffin and segmented into 5 μm thick slices, which were subjected to hematoxylin and eosin staining. These sections were then viewed under a light microscope, and images were captured with an Olympus DP12 camera (Japan) to assess histopathological changes. The pathologist performing the analysis was blinded to the group assignments.

Statistical analysis

All results are reported as mean ± standard error of the mean. Statistical changes between groups were assessed using one-way analysis of variance, followed by the Bonferroni *post hoc* test when applicable, using Prism software (GraphPad). A $P < 0.05$ was acknowledged as statistically meaningful for all analyses.

RESULTS

Effect of Vit D and PIO on glucose, insulin, and HOMA-IR levels in STZ-NA-induced diabetes

In the DC rats, there was a major upturn in glucose, insulin, and HOMA-IR levels when aligned with the NC rats, indicating poor metabolic control typical of diabetes. Treatment with Vit D, PIO, and their combination therapy resulted in considerable reductions in glucose, insulin, and HOMA-IR levels, equated to the untreated diabetic group. This suggests that both Vit D and PIO have a positive impact on regulating these metabolic parameters in diabetes. Notably, the combined treatment of Vit D and PIO led to a greater reduction in these parameters than either treatment alone, with the combination therapy proving more effective than Vit D monotherapy. This indicates a synergistic effect between the two agents, enhancing their overall therapeutic benefit in controlling glucose and insulin homeostasis in diabetic rats [Figure 1].

Effect of Vit D and PIO on serum creatinine and BUN levels in STZ-NA-induced diabetes

In the DC rats, serum creatinine and BUN levels were significantly raised compared to the NC group, indicating kidney stress and potential renal damage associated with

diabetes. However, treatment with Vit D, PIO, and their combination led to significant reductions in both serum creatinine and BUN levels compared to the untreated diabetic group, suggesting a protective effect on kidney function. Among the treatments, the combination of Vit D and PIO resulted in the most substantial decrease in creatinine levels, outperforming Vit D monotherapy. However, the combination did not show a significant improvement in BUN levels compared to Vit D alone, indicating that while the combination therapy was effective in reducing creatinine, its impact on BUN may be less pronounced. This highlights a differential response between the two kidney function markers, suggesting that the combination therapy may exert specific protective effects on certain aspects of renal function in diabetic rats [Figure 2].

Effect of Vit D and PIO on CRP and MCP-1 levels in STZ-NA-induced diabetes

In diabetic rats, there was a substantial elevation in CRP and MCP-1 levels aligned to the NC group. Treatment with Vit D or PIO did not result in any substantial changes in CRP and MCP-1 levels compared to the untreated diabetic group. However, the combined treatment of Vit D and PIO did lead to a noticeable reduction in the levels of both markers when matched with the DC group. It is important to highlight that the combination therapy did not offer any additional benefit over the individual treatments in terms of reducing these inflammatory markers [Figure 3].

Effect of Vit D and PIO on TNF-α, Cystatin-C, IL-6, and TGF-β1 levels in STZ-NA-induced diabetes

In DC rats, there was a marked increase in the levels of inflammatory and fibrotic markers, such as TNF-α, Cystatin-C, IL-6, and TGF-β1, when compared to normal rats. Treatment with Vit D, PIO, or a combination of both led to a considerable drop in these markers compared to the untreated diabetic rats. Notably, the combination of Vit D and PIO resulted in a significantly greater reduction in IL-6 levels than Vit D treatment alone. Yet, there was no considerable difference in the reduction of TNF-α, cystatin-C, or TGF-β1

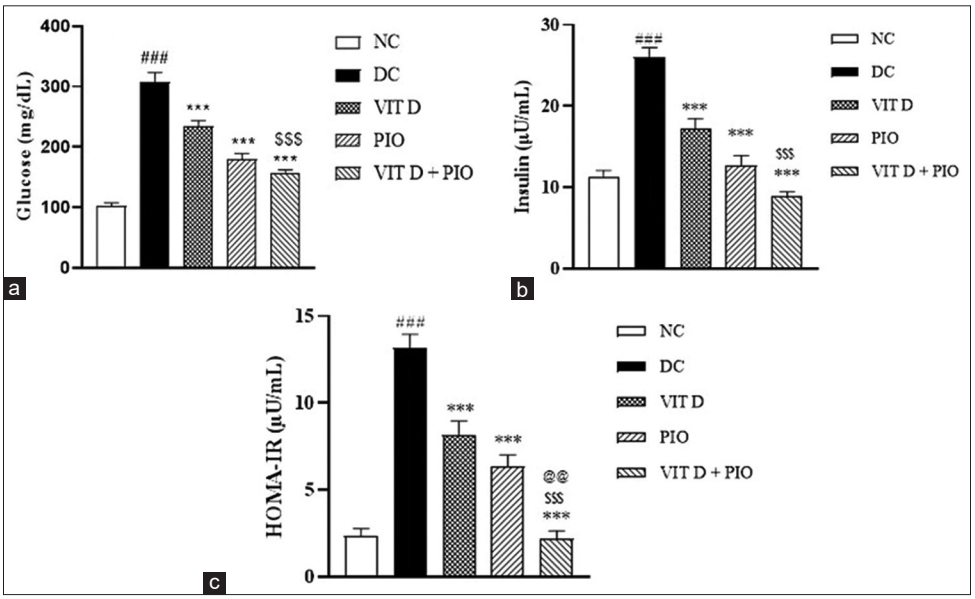

Figure 1: Impact of Vitamin D (Vit D) and pioglitazone (PIO) on (a) glucose (b) insulin and (c) homeostasis model assessment of insulin resistance in experimentally induced diabetes. Values are stated as mean ± standard error of the mean; *n*=6. ###*P*<0.001, relative to normal control; ***P*<0.001, relative to diabetic control; $$$*P*<0.001 relative to Vit D; @@*P*<0.01 relative to PIO

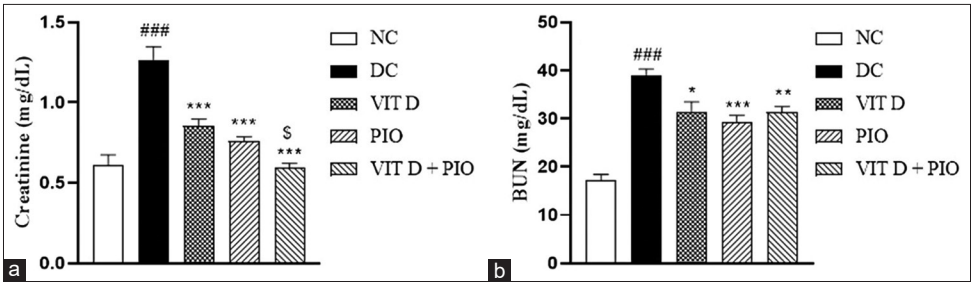

Figure 2: Impact of Vitamin D (Vit D) and pioglitazone on (a) creatinine and (b) Blood urea nitrogen in experimentally induced diabetes. Values are stated as mean ± ± standard error of the mean; *n*=6. ###*P*<0.001, relative to normal control; **P*<0.05, ***P*<0.01, ***P*<0.001 relative to diabetic control; $*P*<0.005 relative to Vit D

levels between the combination therapy and the individual treatments. These findings suggest that while both Vit D and PIO are effective at reducing markers of inflammation and fibrosis in diabetes, their combined effect may specifically reduce IL-6 more efficiently, without providing additional benefits for the other markers when compared to monotherapy [Figure 4].

Histopathology study

The histomorphological analysis of renal tissue across different groups revealed varying levels of changes. In the NC group, the renal parenchyma appeared intact, with no pathological alterations in the renal tubules and glomeruli, apart from localized vascular congestion. In contrast, the DC group displayed mild degenerative changes, including swelling of the tubular epithelium, loss of some epithelial cells, granular cytoplasm in degenerated tubules, atrophic glomeruli, eosinophilic deposits in the tubule lumens, mild vascular congestion, and occasional interstitial hemorrhages. Both the Vit D and PIO treatment groups exhibited minimal degenerative changes, such as cellular swelling, occasional epithelial loss, and granular cytoplasm in degenerated tubules, along with focal vascular congestion, but no signs of inflammation. The combination of Vit D and PIO treatment showed normal renal histomorphology, with intact renal tubules and glomeruli, similar to the NC group, although mild focal vascular congestion remained without any inflammatory changes [Figure 5].

DISCUSSION

The results of this study indicate that both Vit D and PIO have considerable effects on glucose metabolism, kidney function, and inflammatory markers in STZ-NA-induced diabetic rats. When administered separately or together, both treatments led to significant improvements compared to the untreated diabetic group, highlighting their potential as therapeutic agents for managing diabetes and its complications. The combination of Vit D and PIO was particularly effective, especially in regulating glucose and insulin levels, and improving renal function, although their effects on inflammatory markers were more complex.

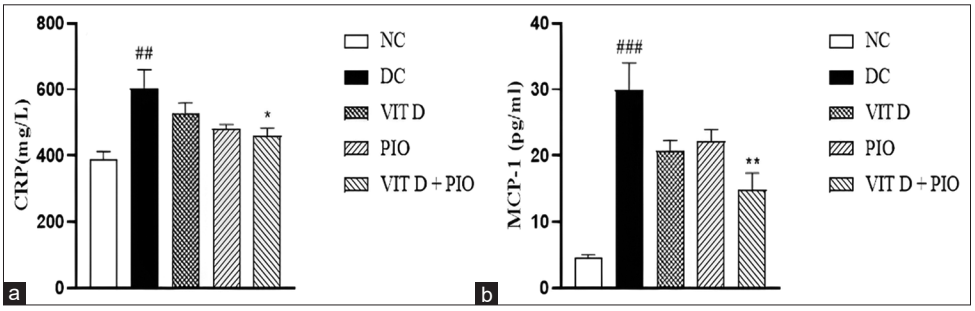

Figure 3: Impact of Vitamin D and pioglitazone on (a) C-reactive protein and (b) monocyte chemoattractant protein-1 in experimentally induced diabetes. Values are stated as mean ± standard error of the mean; *n*=6. ##*P*<0.01, ###*P*<0.001 relative to normal control; **P*<0.05, ***P*<0.01, relative to diabetic control

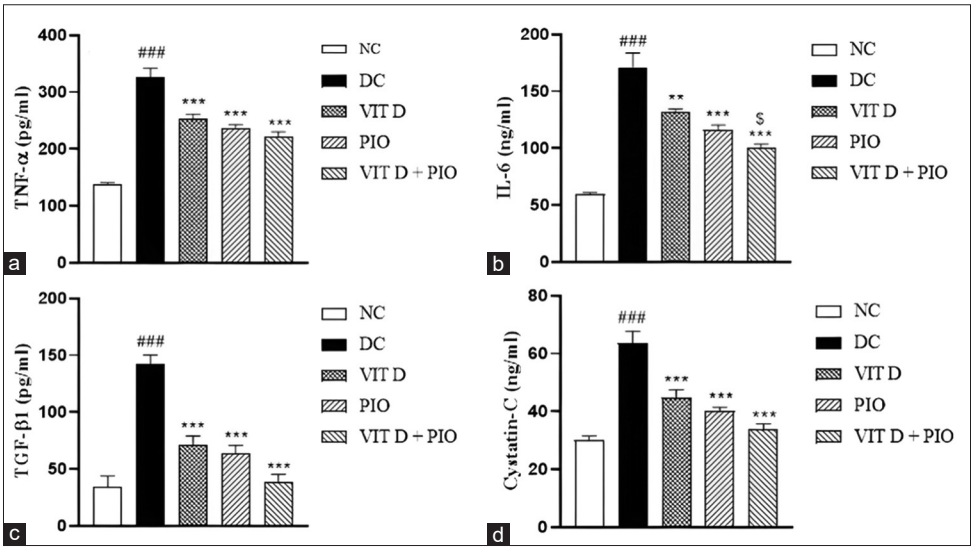

Figure 4: Impact of Vitamin D (Vit D) and pioglitazone on (a) tumor necrosis factor-alpha (b) cystatin-C (c) interleukin-6, and (d) transforming growth factor-beta 1 in experimentally induced diabetes. Values are stated as mean ± standard error of the mean; *n*=6. ###*P*<0.001 relative to normal control; ****P*<0.001, relative to diabetic control; $*P*<0.005 relative to Vit D

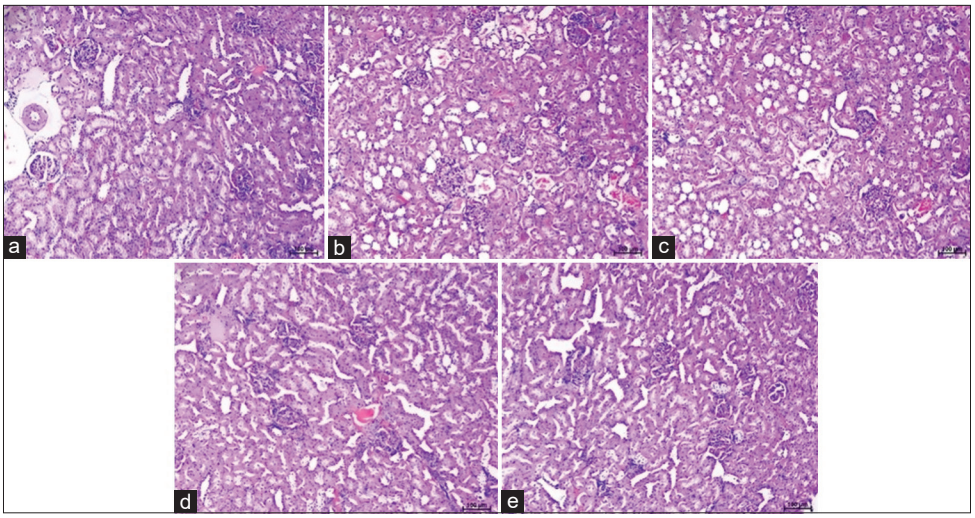

Figure 5: Light microscopy of kidney tissue from rats (a) normal control (b) diabetic control (c) Vitamin D (Vit D) (d) pioglitazone (PIO) and (e) Vit D + PIO

Regarding glucose metabolism, the induction of diabetes in rats resulted in significantly higher levels of glucose, insulin, and HOMA-IR, which is consistent with prior studies that report poor metabolic control in diabetic models.[15] Treatment with Vit D, PIO, and their combination resulted in significant reductions in these parameters, indicating enhanced glucose regulation, which is consistent with findings from previous studies.[16,17] The combination of Vit D and PIO was particularly effective, producing greater reductions than Vit D alone, supporting the idea that Vit D may enhance PIO's effects, as has been observed in other studies.[12]

As for kidney function, elevated serum creatinine and BUN levels, common markers of kidney dysfunction in diabetes,[18,19] were significantly reduced by both Vit D and PIO treatments. These findings align with previous studies that suggest nephroprotective properties of both agents.[20,21] Interestingly, while the combination treatment showed the most significant reduction in creatinine, it did not further decrease BUN levels compared to Vit D alone, indicating that Vit D and PIO may act through distinct mechanisms to influence kidney function. This difference in the effects on creatinine and BUN levels may reflect the distinct roles; these markers play in assessing renal damage.

In our findings, CRP and MCP-1 levels did not show significant changes with either Vit D or PIO monotherapy, which contrasts with earlier studies that reported notable anti-inflammatory effects of these treatments, marked by significant alterations in these biomarkers.[22-25] However, the combined treatment led to a noticeable reduction in both markers, though it did not offer significant additional benefits over individual treatments. This could be due to the specificity of the inflammatory indicators measured; while CRP and MCP-1 are general markers of systemic inflammation. Vit D and PIO may more effectively target other inflammatory cytokines, such as TNF-α and IL-6, in different experimental models.[26,27] Thus, Vit D or PIO may be more pronounced on specific inflammatory pathways rather than the general inflammatory markers assessed here.

Regarding other inflammatory and fibrotic markers, the combination therapy resulted in a significant reduction in IL-6 levels, which supports previous findings that Vit D and PIO can reduce inflammatory cytokines associated with hepatic damage.[12] However, no additional benefit was observed for TNF-α, Cystatin-C, or TGF-β1 levels with the combination treatment than monotherapy, suggesting that these markers may not be as responsive to the synergistic effects of Vit D and PIO. TGF-β1, a key marker of fibrosis, has been shown to be reduced by both agents in DN,[8,28] but the current study did not find significant differences between monotherapy and combination therapy, possibly due to the specific timing of treatment or the need for longer treatment periods in later stages of DN.

Histopathological examination revealed that both Vit D and PIO treatments provided partial protection against renal damage, such as tubular degeneration and glomerular atrophy, with the combination therapy showing near-normal renal structure. This is consistent with other studies that report protective effects of Vit D and PIO on DN by alteration in histological characteristics.[20,21] The absence of significant inflammation in the combination treatment group suggests that the two agents may work synergistically to preserve renal tissue integrity, potentially through the modulation of inflammatory pathways.

CONCLUSION

The combination of Vit D and PIO demonstrated enhanced therapeutic benefits in STZ-NA-induced diabetic rats, improving metabolic control, kidney function, and inflammatory markers. The synergistic effect of Vit D and PIO resulted in greater reductions in glucose, insulin, and

HOMA-IR levels compared to monotherapy, suggesting superior metabolic regulation. While both treatments effectively reduced serum creatinine and BUN levels, the combination notably lowered creatinine more significantly, offering robust renal protection. Inflammatory markers such as CRP, MCP-1, and IL-6 were reduced with the combination therapy, although no additional benefit was seen over individual treatments for other markers. Histopathologically, the combination therapy restored kidney structure to near normal, with minimal vascular congestion. These findings highlight the potential of combined Vit D and PIO therapy as a more effective strategy for treating diabetes and related kidney issues.

REFERENCES

1. Kumar M, Dev S, Khalid MU, Siddenthis SM, Noman M, John C, *et al*. The bidirectional link between diabetes and kidney disease: Mechanisms and management. Cureus 2023;15:e45615.
2. Rayego-Mateos S, Morgado-Pascual JL, Opazo-Ríos L, Guerrero-Hue M, García-Caballero C, Vázquez-Carballo C, *et al*. Pathogenic pathways and therapeutic approaches targeting inflammation in diabetic nephropathy. Int J Mol Sci 2020;21:3798.
3. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 2008;19:433-42.
4. El Mesallamy HO, Ahmed HH, Bassyouni AA, Ahmed AS. Clinical significance of inflammatory and fibrogenic cytokines in diabetic nephropathy. Clin Biochem 2012;45:646-50.
5. Jeon YK, Kim MR, Huh JE, Mok JY, Song SH, Kim SS, *et al*. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. J Korean Med Sci 2011;26:258-63.
6. Sao RK, Gupta M, Bachu L. Study of diabetic nephropathy in relation with cystatin-C level and inflammatory markers (hs-CRP) in type-2 diabetes mellitus patients. Eur J Mol Clin Med 2021;8:2380-9.
7. Nakashima A, Yokoyama K, Yokoo T, Urashima M. Role of vitamin D in diabetes mellitus and chronic kidney disease. World J Diabetes 2016;7:89-100.
8. Tian Y, Lv G, Yang Y, Zhang Y, Yu R, Zhu J, *et al*. Effects of vitamin D on renal fibrosis in diabetic nephropathy model rats. Int J Clin Exp Pathol 2014;7:3028-37.
9. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. Nutrients 2020;12:575.
10. Lei M, Liu Z, Guo J. The emerging role of vitamin D and vitamin D receptor in diabetic nephropathy. Biomed Res Int 2020;2020:4137268.
11. Hu X, Liu W, Yan Y, Liu H, Huang Q, Xiao Y, *et al*. Vitamin D protects against diabetic nephropathy: Evidence-based effectiveness and mechanism. Eur J Pharmacol 2019;845:91-8.
12. Hamouda HA, Mansour SM, Elyamany MF. Vitamin D combined with pioglitazone mitigates type-2 diabetes-induced hepatic injury through targeting inflammation, apoptosis, and oxidative stress. Inflammation 2022;45:156-71.
13. Alsolami KM. Potential protective effect of vitamin D on cardiac apoptosis in obese rats. Genet Mol Res 2019;18:gmr16039950.
14. Ding SY, Shen ZF, Chen YT, Sun SJ, Liu Q, Xie MZ. Pioglitazone can ameliorate insulin resistance in low-dose streptozotocin and high sucrose-fat diet induced obese rats. Acta Pharmacol Sin 2005;26:575-80.
15. Cai S, Sun W, Fan Y, Guo X, Xu G, Xu T, *et al*. Effect of mulberry leaf (Folium Mori) on insulin resistance via IRS-1/PI3K/Glut-4 signalling pathway in type 2 diabetes mellitus rats. Pharm Biol 2016;54:2685-91.
16. Mostafa DK, Nasra RA, Zahran N, Ghoneim MT. Pleiotropic protective effects of Vitamin D against high fat diet-induced metabolic syndrome in rats: One for all. Eur J Pharmacol 2016;792:38-47.
17. Refaat R, Sakr A, Salama M, El Sarha A. Combination of vildagliptin and pioglitazone in experimental type 2 diabetes in male rats. Drug Dev Res 2016;77:300-9.
18. Maheshwari RA, Balaraman R, Sen AK, Seth AK. Effect of coenzyme Q10 alone and its combination with metformin on streptozotocin-nicotinamide-induced diabetic nephropathy in rats. Indian J Pharmacol 2014;46:627-32.
19. Hu W, Feng P. Elevated serum chemerin concentrations are associated with renal dysfunction in type 2 diabetic patients. Diabetes Res Clin Pract 2011;91:159-63.
20. Wang H, Wang J, Qu H, Wei H, Ji B, Yang Z, *et al*. *In vitro* and *in vivo* inhibition of mTOR by 1, 25-dihydroxyvitamin D_3 to improve early diabetic nephropathy via the DDIT4/TSC2/mTOR pathway. Endocrine 2016;54:348-59.
21. Peng XH, Liang PY, Ou SJ, Zu XB. Protective effect of pioglitazone on kidney injury in diabetic rats. Asian Pac J Trop Med 2014;7:819-22.
22. Jain SK, Micinski D. Vitamin D upregulates glutamate cysteine ligase and glutathione reductase, and GSH formation, and decreases ROS and MCP-1 and IL-8 secretion in high-glucose exposed U937 monocytes. Biochem Biophys Res Commun 2013;437:7-11.
23. Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: A meta-analysis of randomized controlled trials. Nutrients 2014;6:2206-16.
24. Heliövaara MK, Herz M, Teppo AM, Leinonen E, Ebeling P. Pioglitazone has anti-inflammatory effects in patients with Type 2 diabetes. J Endocrinol Invest 2007;30:292-7.
25. Forst T, Karagiannis E, Lübben G, Hohberg C, Schöndorf T, Dikta G, *et al*. Pleiotrophic and anti-inflammatory effects of pioglitazone precede the metabolic activity in type 2 diabetic patients with

coronary artery disease. Atherosclerosis 2008;197:311-7.
26. Kim CH, Ahn JW, You RM, Kim SH, Chae HD, Kang BM. Pioglitazone treatment decreases follicular fluid levels of tumor necrosis factor-α and interleukin-6 in patients with polycystic ovary syndrome. Clin Exp Reprod Med 2011;38:98-102.
27. Esfandiari A, Gargari BP, Noshad H, Sarbakhsh P, Mobasseri M, Barzegari M, *et al*. The effects of vitamin D3 supplementation on some metabolic and inflammatory markers in diabetic nephropathy patients with marginal status of vitamin D: A randomized double blind placebo controlled clinical trial. Diabetes Metab Syndr 2019;13:278-83.
28. Ko GJ, Kang YS, Han SY, Lee MH, Song HK, Han KH, *et al*. Pioglitazone attenuates diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. Nephrol Dial Transplant 2008;23:2750-60.

Source of Support: Nil. **Conflicts of Interest:** None declared.