Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2024: 84.28
Volume 15, Issue 2; 2026, 41-50

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Research Article

Renoprotective Effect of Dapagliflozin and Valproic Acid on STZ Induce Diabetic Nephropathy in Wister Rats

Himanshu Peshwani[1], Divya Singh[2], Rakesh Sharma[3], Lokesh Kumar Saini[4], Ashutosh Sharma[5], Aziz Ahmed[6]

[1]Research Scholar, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur (Rajasthan) India
[2,3,4]Department of Pharmacology, Jaipur College of Pharmacy, Jaipur (Rajasthan) India
[5]Department of Pharmaceutics, Jaipur College of Pharmacy, Jaipur (Rajasthan) India
[6]Department of Pharmacognosy, Jaipur College of Pharmacy, Jaipur (Rajasthan) India

Article Info: Received: 17-02-2026 / Revised: 04-03-2026 / Accepted: 28-03-2026
Corresponding Author: Himanshu Peshwani
DOI: https://doi.org/10.32553/jbpr.v15i2.1444
Conflict of interest statement: No conflict of interest

Abstract:
Diabetic nephropathy (DN) is a major microvascular complication of diabetes mellitus, leading to chronic kidney disease and end-stage renal failure. Current therapies only slow progression but do not reverse renal damage. The present study was designed to evaluate the renoprotective potential of dapagliflozin (SGLT2 inhibitor) and valproic acid (HDAC inhibitor with antioxidative and anti-inflammatory activity), individually and in combination, in streptozotocin (STZ)-induced diabetic nephropathy in Wistar rats. Experimental diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg). Animals were divided into six groups: normal control, diabetic control, standard drug-treated, dapagliflozin-treated, valproic acid-treated, and combination-treated groups. Treatments were continued for 8 weeks. Parameters assessed included fasting blood glucose, body weight, renal function markers (serum creatinine, urea, uric acid, BUN), oxidative stress markers (SOD, CAT, GSH, MDA), inflammatory cytokines, and histopathological examination of renal tissue. Results showed significant renal impairment in diabetic control rats, with elevated serum creatinine, urea, BUN, and marked histopathological changes. Dapagliflozin and valproic acid treatment significantly improved renal parameters, attenuated oxidative stress, and preserved histological architecture. Combination therapy demonstrated greater renoprotective efficacy compared to monotherapy. The study concludes that dapagliflozin and valproic acid possess complementary renoprotective actions, suggesting potential for combination therapy in diabetic nephropathy management.
Keywords: Diabetic nephropathy, dapagliflozin, valproic acid, Wistar rats, oxidative stress, renoprotection.

Introduction

The kidney is an important organ prerequisite by the body to perform several essential regulatory roles including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs. Therefore, the kidney can be considered as a vital target tissue for exogenous toxicants. Nephrotoxicity refers to

dangerous kidney problems that develop when toxins accumulate in the kidneys.

Toxins and unneeded fluids are usually excreted through urine, but when they start reaching excessive levels, they eventually lead to nephrotoxicity and cause a variety of other symptoms of kidney trouble. It can lead to acute renal failure, in which the kidneys suddenly lose their ability to function, or chronic renal failure, in which kidney function slowly deteriorates.[1] If unchecked, renal failure can result in death.

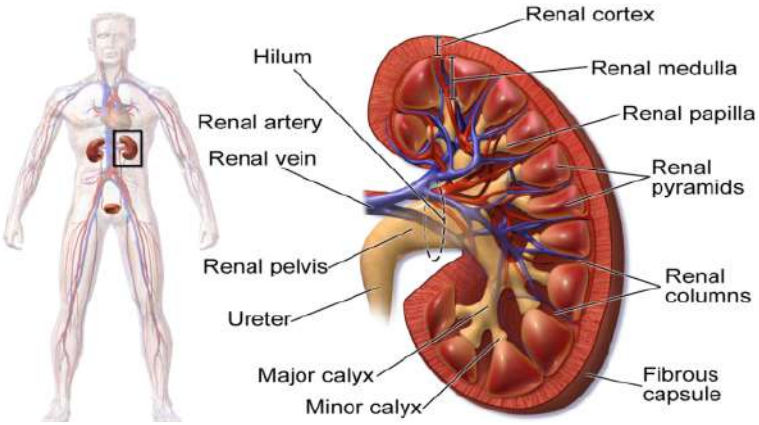


Figure 1: Anatomy of Kidney

Nephrotoxicity occur when kidney excretion rate or destruction of kidney function by exogenous and endogenous toxic substance becomes low. Also diabetes mellitus (DM) is well- known endocrine disease induced via acquired or inherited lack of insulin production in pancreas tissue. According to the report, 300 million people are affected by DM until 2025.

Diabetes mellitus (DM) is a chronic, heterogeneous metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

The condition is associated with disturbances in carbohydrate, fat, and protein metabolism, which over time lead to chronic complications affecting the eyes, kidneys, nerves, and cardiovascular system.[2]

Materials and Methods

Chemicals and Drugs:

The following chemicals and drugs were employed in the present investigation:[3]

- **Streptozotocin (STZ):** Procured from Oswal-Scientific store, India used for the induction of experimental diabetes in Wistar rats.
- **Dapagliflozin:** Obtained from Local market used as a sodium–glucose co-transporter 2 (SGLT2) inhibitor for treatment groups.
- **Valproic Acid:** Procured from Local market used as a histone deacetylase (HDAC) inhibitor with antioxidant and anti-inflammatory activity.
- **Standard Drug – Glibenclamide:** Procured from industry used as a reference antidiabetic drug for comparison.

Experimental animals:

Wistar albino rats of either sex, weighing 200-250g will be employed in the present study. They will be fed on standard chow diet. Food and water will be provided ad libitium throughout experimental period.

They will be housed in departmental animal house and will be exposed to 12h light and 12h dark cycles. All animals will be maintained as per the CCSEA guidelines for the care and use of Laboratory Animals, the experimental protocol used in the present study will be approved by Institutional Animal Ethics Committee.[4]

Induction of Diabetes:

Diabetes was experimentally induced in overnight-fasted Wistar rats by administering a single intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight. STZ was freshly dissolved in 0.1 M cold citrate buffer (pH 4.5) immediately prior to injection to maintain stability, as it is known to degrade rapidly in aqueous solutions.[5]

Experimental Groups

Table 1: Grouping, Dose and Route in Animal

Group No.	Group name / Treatment	Dose (mg/kg)	No. of Animals	Route
I	Normal control (Vehicle)	Vehicle (0.5% CMC or appropriate)	6	p.o.
II	Diabetic control (STZ)	55mg/kg	6	i.p.
III	STZ + Dapagliflozin	1 mg/kg	6	p.o.
IV	STZ + Valproic acid (VPA)	200 mg/kg	6	p.o.
V	STZ + Dapagliflozin + VPA	Dapagliflozin 1 mg/kg + VPA 200 mg/kg	6	p.o.
VI	STZ + Standard (Glibenclamide)	5 mg/kg	6	p.o.

Parameters Evaluated

Biochemical Parameters: Biochemical estimations were carried out to assess the effect of dapagliflozin and valproic acid on renal function, glucose homeostasis, and lipid metabolism in STZ-induced diabetic rats.[6-10]

a) Blood Glucose

b) Serum Creatinine

c) Blood Urea Nitrogen (BUN) and Urea

d) Uric Acid

e) Lipid Profile

Biochemical Parameters Evaluated and Their Significance

Table 2: Biochemical Parameters Evaluated and Their Significance

Parameter	Method/Kit Used	Significance in Diabetic Nephropathy
Blood glucose	Glucometer (tail vein)	Confirms diabetic status; measures antihyperglycemic effect
Serum creatinine	Jaffe's method	Marker of GFR and renal function
BUN/Urea	Urease method	Reflects renal clearance and protein catabolism
Uric acid	Uricase method	Marker and mediator of oxidative stress and endothelial dysfunction
Lipid profile (TC, TG, LDL, HDL)	Enzymatic colorimetric assay	Assesses dyslipidemia and risk of macrovascular/microvascular complications

Oxidative Stress Parameters: Oxidative stress plays a central role in the development and progression of diabetic nephropathy. Hyperglycemia leads to excessive generation of reactive oxygen species (ROS), overwhelming the antioxidant defense system, thereby causing lipid peroxidation, protein oxidation, and DNA damage. Renal tissues were homogenized in appropriate buffers and assayed for oxidative stress parameters as follows:[11,12]

a) Malondialdehyde (MDA)

b) Reduced Glutathione (GSH)

c) Superoxide Dismutase (SOD)

d) Catalase (CAT)

Oxidative Stress Parameters Evaluated in Renal Tissue

Table 3: Oxidative Stress Parameters Evaluated in Renal Tissue

Parameter	Unit	Normal Range (Rat Kidney)	Method
Malondialdehyde (MDA)	nmol/mg protein	0.5–1.5	TBARS assay
Reduced Glutathione (GSH)	µmol/mg protein	20–35	Ellman's DTNB method
Superoxide Dismutase (SOD)	U/mg protein	5–10	Epinephrine auto-oxidation inhibition
Catalase (CAT)	U/mg protein	40–70	H_2O_2 decomposition assay

Inflammatory Markers

Chronic hyperglycemia induces a pro-inflammatory state that plays a crucial role in the progression of diabetic nephropathy. Elevated levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) contribute to oxidative stress, apoptosis, endothelial dysfunction, and renal fibrosis. These cytokines were quantified to assess the anti-inflammatory potential of dapagliflozin and valproic acid.[13,14]

a) Tumor Necrosis Factor-α (TNF-α)
b) Interleukin-6 (IL-6)

Inflammatory Markers Evaluated in Serum and Renal Tissue

Table 4: Inflammatory Markers Evaluated in Serum and Renal Tissue

Marker	Method	Unit	Normal Range (Rat Serum)
TNF-α	ELISA kit	pg/mL	10–50 pg/mL
IL-6	ELISA kit	pg/mL	15–80 pg/mL

Histopathology of Kidney

Histopathological evaluation provides direct evidence of renal structural alterations in diabetic nephropathy and helps to correlate biochemical changes with tissue damage.

Kidneys were excised at the end of the experiment and processed for histological analysis using Hematoxylin and Eosin (H&E) staining**,** the most widely employed staining method in experimental nephrology.[15,16]

Procedure:

Kidneys were excised, rinsed in ice-cold normal saline to remove blood, and fixed in 10% neutral buffered formalin for at least 24 h. Tissue samples were dehydrated in ascending grades of ethanol (70%, 90%, and absolute), cleared in xylene, and embedded in paraffin wax.

Paraffin blocks were sectioned into 4–5 µm thick slices using a rotary microtome. Sections were mounted on glass slides and subjected to H&E staining: Hematoxylin stains cell nuclei blue/purple. Eosin counterstains cytoplasm and extracellular proteins pink. Stained slides were mounted with DPX (Distrene Plasticizer Xylene) and examined under a light microscope (Olympus, Japan) at 40× magnification.

Photomicrographs were captured for documentation.

At the end of the experimental period (8 weeks of treatment), animals from all groups were sacrificed under mild anesthesia, and both kidneys were excised for histopathological examination.

Tissue collection and fixation: Kidneys were removed, washed gently with ice-cold saline to eliminate blood, and immediately fixed in 10% neutral buffered formalin for 24–48 h.

Statistical Analysis:

All experimental results were expressed as Mean ± Standard Error of Mean (SEM) for each group (n = 6). Statistical evaluation was performed to determine the significance of differences among experimental groups.

Results

Effect on Blood Glucose Levels

Administration of STZ (55 mg/kg, i.p.) produced significant hyperglycemia compared to normal control rats. Diabetic control animals maintained persistently high fasting blood glucose (FBG) levels throughout the study.

Table 5: Effect of Dapagliflozin and Valproic Acid on Fasting Blood Glucose Levels (mg/dL) in STZ-Induced Diabetic Rats (Mean ± SEM, n=6)

Group	FBG after STZ induction (mg/dL)	FBG after 8 weeks (mg/dL)
I – Normal Control	92.3 ± 4.1	95.6 ± 3.8
II – Diabetic Control	282.4 ± 10.7	310.8 ± 12.2
III – Dapagliflozin (1 mg/kg)	285.7 ± 9.8	162.3 ± 8.9 **
IV – Valproic Acid (200 mg/kg)	288.9 ± 11.3	178.6 ± 9.2 **
V – Dapa + VPA	289.6 ± 10.5	132.4 ± 7.8 ***
VI – Glibenclamide (5 mg/kg)	286.1 ± 9.7	125.8± 6.9 ***

Significance: p<0.01 vs. Diabetic Control (**), p<0.001 vs. Diabetic Control (***)

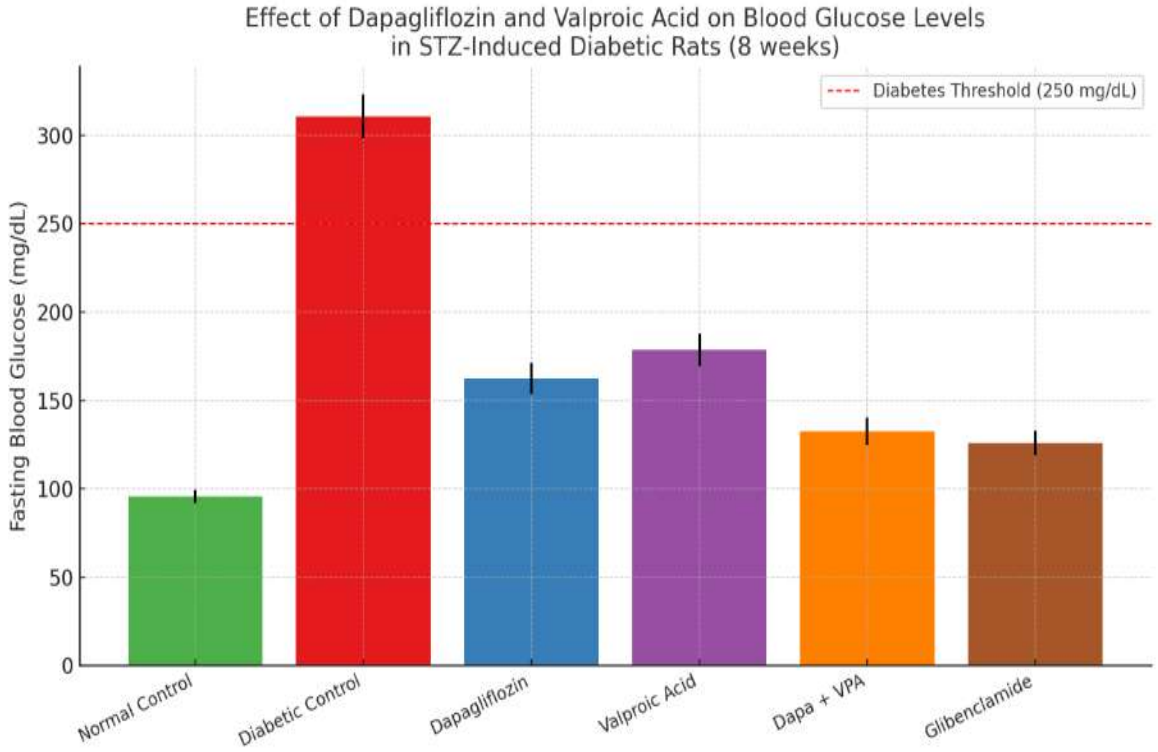


Figure 2: Effect of Dapagliflozin and Valproic Acid on Fasting Blood Glucose Levels (mg/dL) in STZ-Induced Diabetic Rats

These findings demonstrate that dapagliflozin and valproic acid, especially in combination, significantly reduce hyperglycemia in STZ-induced diabetic rats, validating their therapeutic potential.

Effect on Serum Renal Function Markers: Diabetic control rats showed significantly elevated serum creatinine, urea, BUN, and uric acid compared to normal controls, indicating renal dysfunction.

Table 6: Effect of Dapagliflozin and Valproic Acid on Renal Function Markers Levels (mg/dL) in STZ-Induced Diabetic Rats (Mean ± SEM, n=6)

Group	FBG after STZ induction (mg/dL)	FBG after 8 weeks (mg/dL)
I – Normal Control	92.3 ± 4.1	95.6 ± 3.8
II – Diabetic Control	282.4 ± 10.7	310.8 ± 12.2
III – Dapagliflozin (1 mg/kg)	285.7 ± 9.8	162.3 ± 8.9 **
IV – Valproic Acid (200 mg/kg)	288.9 ± 11.3	178.6 ± 9.2 **
V – Dapa + VPA	289.6 ± 10.5	132.4 ± 7.8 ***
VI – Glibenclamide (5 mg/kg)	286.1 ± 9.7	125.8± 6.9 ***

Significance: p<0.01 vs. Diabetic Control (**), p<0.001 vs. Diabetic Control (***)

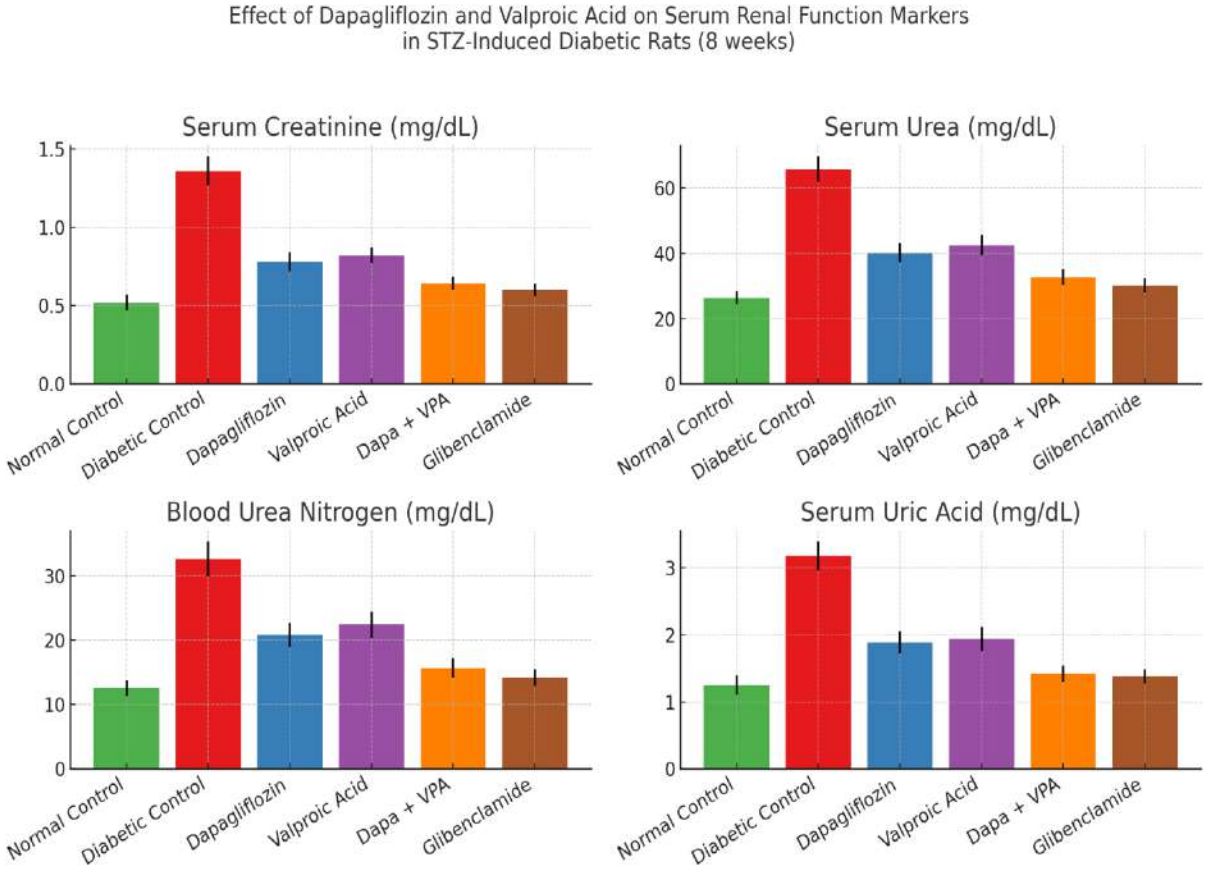


Figure 3: Effect of Dapagliflozin and Valproic Acid on Fasting Renal Function Markers in STZ-Induced Diabetic Rats

These findings demonstrate that dapagliflozin and valproic acid, especially in combination, significantly reduce hyperglycemia in STZ-induced diabetic rats, validating their therapeutic potential.

Treatment with dapagliflozin and valproic acid individually attenuated these changes, while the combination showed maximal renoprotective effect, comparable to glibenclamide.

Effect on Oxidative Stress Parameters (Renal Tissue): Diabetic control rats exhibited increased MDA levels and significantly reduced GSH, SOD, and CAT activities, confirming oxidative stress.

Table 7: Effect of Dapagliflozin and Valproic Acid on Oxidative Stress Parameters in Renal Tissue (Mean ± SEM, n = 6)

Group	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
I – Normal Control	1.2 ± 0.1	30.5 ± 2.0	9.2 ± 0.6	65.3 ± 3.5
II – Diabetic Control	3.8 ± 0.2 ###	14.2 ± 1.5 ###	4.1 ± 0.5 ###	32.6 ± 2.8 ###
III – Dapagliflozin	2.3 ± 0.2 **	22.4 ± 1.7 **	6.8 ± 0.5 **	49.5 ± 3.0 **
IV – Valproic Acid	2.4 ± 0.2 **	23.1 ± 1.6 **	7.0 ± 0.6 **	50.4 ± 3.1 **
V – Dapa + VPA	1.6 ± 0.1 ***	27.8 ± 1.8 ***	8.3 ± 0.4 ***	59.2 ± 2.7 ***
VI – Glibenclamide	1.5 ± 0.1 ***	28.4 ± 1.7 ***	8.5 ± 0.5 ***	60.1± 2.8 ***

Significance: p<0.001 vs. Normal Control, ** = p<0.01 vs. Diabetic Control, *** = p<0.001 vs. Diabetic Control

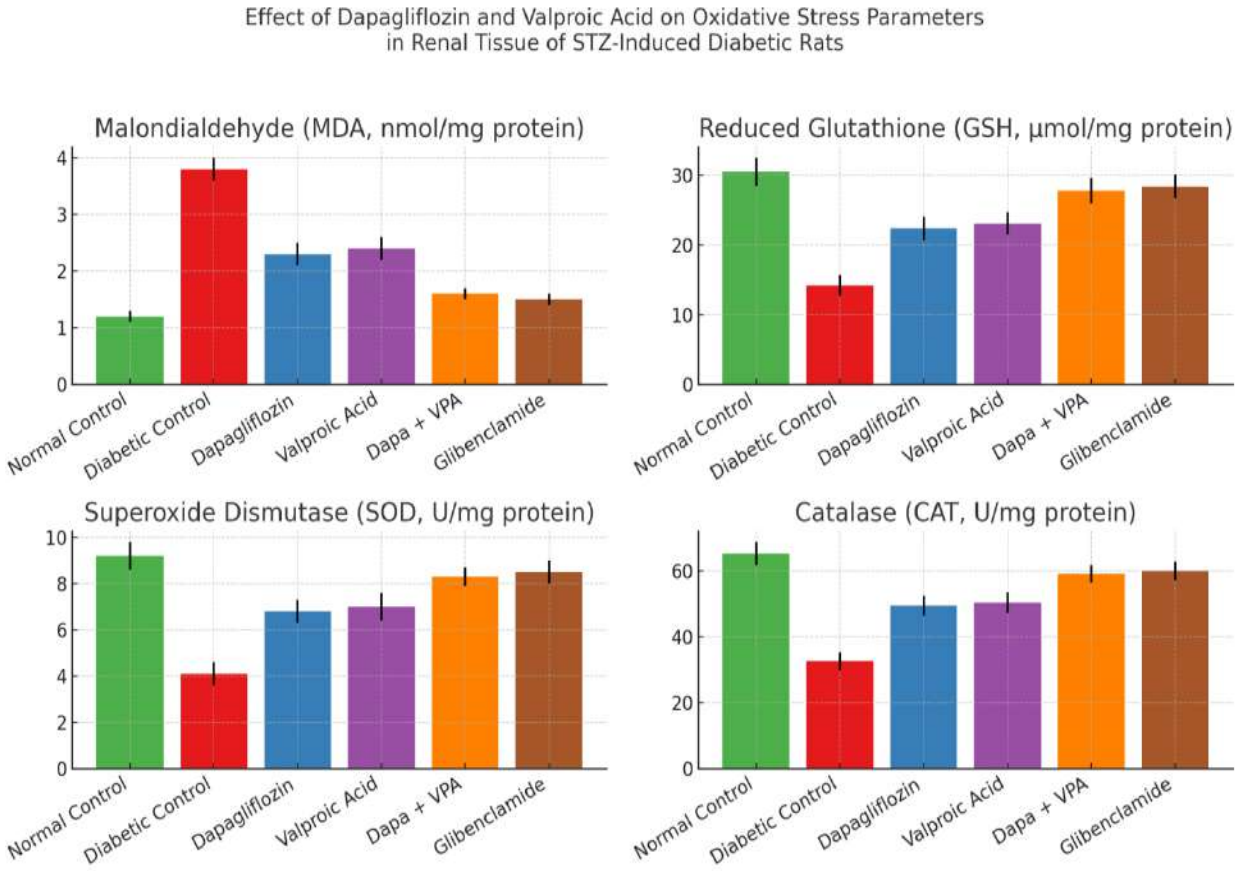


Figure 4: Effect of Dapagliflozin and Valproic Acid on Oxidative Stress Parameters in Renal Tissue

These findings suggest that dapagliflozin and valproic acid alleviate oxidative stress in diabetic nephropathy, with maximum benefit in combination therapy.

Dapagliflozin and valproic acid treatment improved antioxidant status. The combination group restored levels closer to normal, showing significant protection against lipid peroxidation.

Effect on Inflammatory Markers (Serum and Renal Tissue): Levels of TNF-α and IL-6 were markedly elevated in diabetic control rats, reflecting systemic and renal inflammation.

Table 8: Effect of Dapagliflozin and Valproic Acid on Inflammatory Markers in STZ-Induced Diabetic Rats (Mean ± SEM, n = 6)

Group	TNF-α (pg/mL)	IL-6 (pg/mL)
I – Normal Control	28.5 ± 2.5	42.3 ± 3.2
II – Diabetic Control	96.4 ± 5.1 ###	138.2 ± 6.7 ###
III – Dapagliflozin	55.2 ± 3.8 **	78.6 ± 4.8 **
IV – Valproic Acid	58.1 ± 3.9 **	82.4 ± 5.0 **
V – Dapa + VPA	39.6 ± 2.9 ***	56.7 ± 3.9 ***
VI – Glibenclamide	36.8 ± 3.0 ***	54.2± 4.1 ***

Significance: = p<0.001 vs. Normal Control, ** = p<0.01 vs. Diabetic Control, *** = p<0.001 vs. Diabetic Control

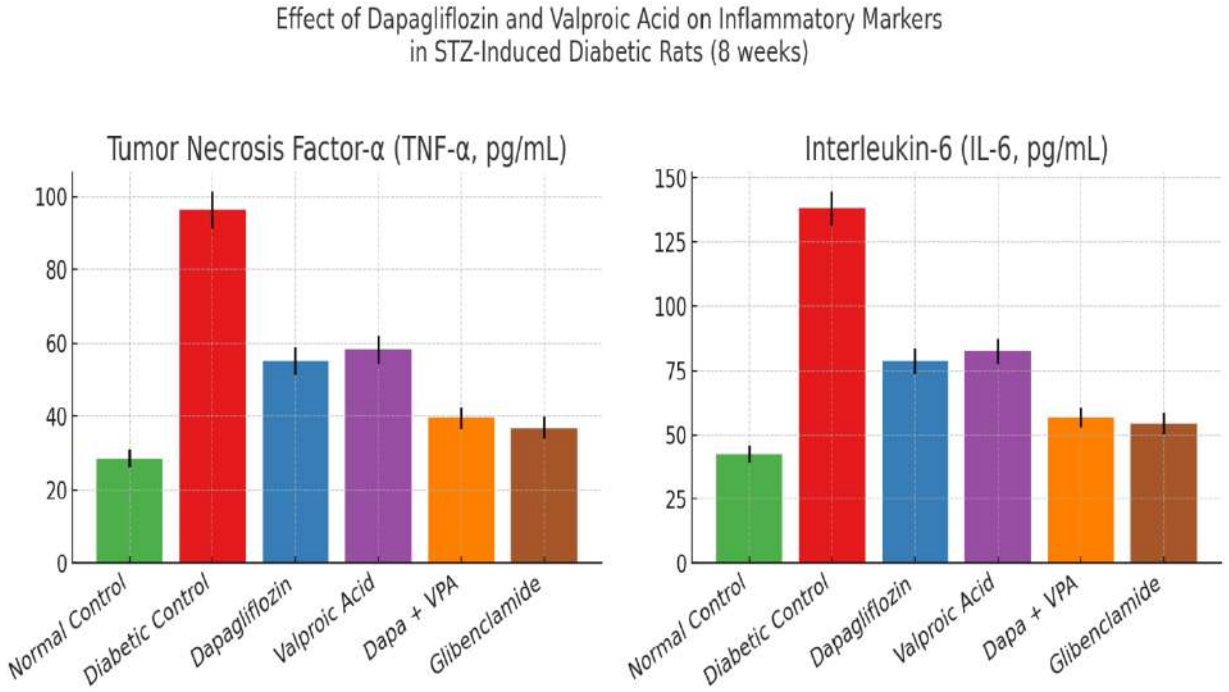


Figure 5: Effect of Dapagliflozin and Valproic Acid on Inflammatory Markers in STZ-Induced Diabetic Rats

These results demonstrate that dapagliflozin and valproic acid, especially in combination, exert potent anti-inflammatory effects that contribute to renal protection in diabetic nephropathy.

Both test drugs significantly reduced cytokine levels ($p<0.01$), with maximum reduction observed in the combination group, comparable to glibenclamide.

Histopathological Examination of Kidney

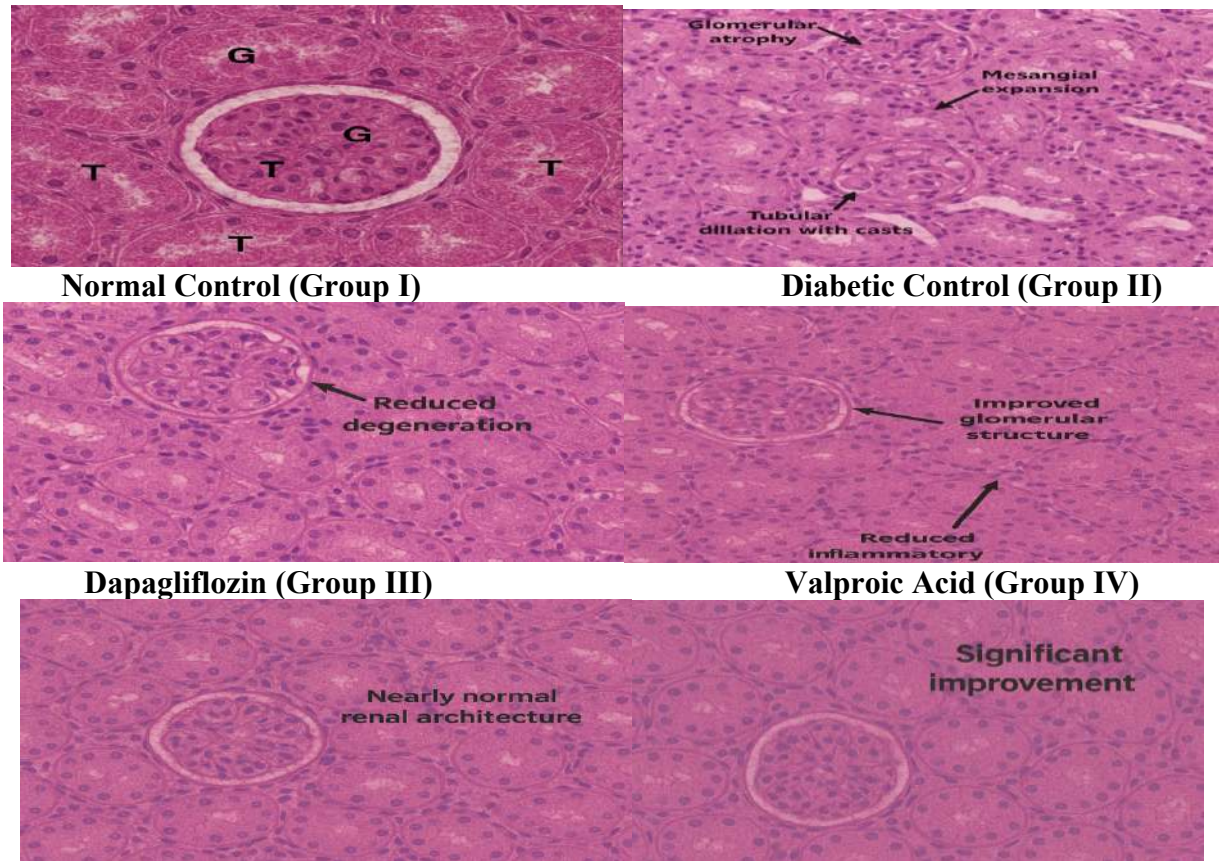
Histopathological evaluation of renal tissue revealed significant differences between groups. Kidneys of normal control rats (Group I) showed intact histoarchitecture with normal glomeruli and tubules. In contrast, diabetic control rats (Group II) displayed characteristic lesions of diabetic nephropathy, including glomerular hypertrophy, mesangial expansion, tubular degeneration, and interstitial inflammation.

Treatment with dapagliflozin (Group III) and valproic acid (Group IV) resulted in noticeable improvement in renal morphology, whereas the combination therapy group (Group V) demonstrated the most prominent protection, with architecture nearly comparable to the standard drug glibenclamide group (Group VI).

Table 12: Semi-Quantitative Histopathological Scores of Renal Lesions (0–4 Scale, Mean ± SEM, n = 6)

Group	Glomerular Hypertrophy	Tubular Degeneration	Interstitial Inflammation	Total Damage Score
I – Normal Control	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.5 ± 0.1
II – Diabetic Control	3.1 ± 0.2 ###	2.9 ± 0.3 ###	2.7 ± 0.2 ###	14.8 ± 0.6 ###
III – Dapagliflozin	1.8 ± 0.2 **	1.7 ± 0.2 **	1.4 ± 0.1 **	8.1 ± 0.5 **
IV – Valproic Acid	1.7 ± 0.3 **	1.6 ± 0.2 **	1.5 ± 0.2 **	7.8 ± 0.6 **
V – Dapa + VPA	0.8 ± 0.1 ***	0.7 ± 0.1 ***	0.6 ± 0.1 ***	3.5 ± 0.3 ***
VI – Glibenclamide	0.9 ± 0.1 ***	0.8 ± 0.1 ***	0.7 ± 0.1 ***	4.0 ± 0.4 ***

Scoring system: 0 = absent; 1 = minimal; 2 = mild; 3 = moderate; 4 = severe. Significance: = p<0.001 vs. Normal Control, ** = p<0.01 vs. Diabetic Control, *** = p<0.001 vs. Diabetic Control



Normal Control (Group I) **Diabetic Control (Group II)**

Dapagliflozin (Group III) **Valproic Acid (Group IV)**

Combination (Group V) **Glibenclamide (Group VI)**

Figure 6: Histopathological Scores of Renal Lesions with All Type of Groups

These findings confirm that dapagliflozin and valproic acid protect against renal injury, with their combination exerting synergistic renoprotective effects comparable to glibenclamide**.**

Discussion

The present study evaluated the renoprotective potential of dapagliflozin (SGLT2 inhibitor) and valproic acid (HDAC inhibitor/anticonvulsant), alone and in combination, in streptozotocin (STZ)-induced diabetic nephropathy (DN) in Wistar rats.

These findings are consistent with earlier reports that STZ selectively destroys pancreatic β-cells, leading to insulin deficiency, chronic hyperglycemia, and subsequent microvascular

complications such as DN. Histopathological improvements, including reduced mesangial expansion and tubular injury, further support its protective effect against DN progression. In this study, VPA significantly reduced TNF-α and IL-6, restored antioxidant enzyme activity, and alleviated renal histopathological injury. These findings indicate that epigenetic regulation via HDAC inhibition plays a role in ameliorating DN. The combination therapy demonstrated synergistic Reno protection, with the most significant improvements across all biochemical, oxidative, inflammatory, and histopathological parameters.

Conclusion

The findings of this study strongly suggest that dapagliflozin and valproic acid, especially in combination, may serve as a promising therapeutic strategy for diabetic nephropathy.

Their complementary mechanisms of action dapagliflozin via glycemic/hemodynamic control and valproic acid via epigenetic, antioxidant, and anti-inflammatory effects — represent a novel, multi-targeted approach for managing DN. Future translational research is warranted to confirm these preclinical findings and pave the way toward clinical application in diabetic patients at risk of nephropathy.

Reference

1. Abdul- Ferguson MA, Vaidya VS and Bonventre JV. (2008) Biomarkers of nephrotoxic acute kidney injury. Toxicology 2008; 245: 182-193.
2. Kennedy N: Kidney problems and Nephrotoxicity. JAPI 2010; 51: 970-979.
3. Singh NP, Ganguli A and Prakash A: Nephrotoxicity. JAPI 2003; 51: 970-979.
4. Kim SY and Moon A: Drug-induced nephrotoxicity and its biomarkers. Biomol Ther (Seoul) 2012; 20: 268-272.
5. Nasiry D, Khalatbary AR, Ahmadvand H, et al. Protective effects of methanolic extract of Juglans regia L. leaf on streptozotocin-induced diabetic peripheral neuropathy in rats. BMC Complement Altern Med. 2017;17:476.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37 Suppl 1:S81–90.
7. World Health Organization. Global report on diabetes. Geneva: WHO; 2016.
8. International Diabetes Federation. IDF Diab etes Atlas. 10th ed. Brussels: IDF; 2021.
9. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, et al. Prevalence of diabetes and prediabetes in 15 states of India: Results from the ICMR-INDIAB population-based cross-sectional study. Lancet Diabetes Endocrinol. 2017;5(8):585–96.
10. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88–98.
11. Rakieten N, Rakieten ML, Nadkarni MR. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother Rep. 1963;29:91–98.
12. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216–226.
13. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52(4):313–20.
14. Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. Science. 1999;285(5432):1390–93.
15. Gawandi SA, Kantharia ND, Tandel KR, Patel CJ. Estimation of serum urea and creatinine in type-2 diabetes mellitus patients. Int J Biomed Adv Res. 2014;5(10):499–502.
16. Johnson RJ, Nakagawa T, Jalal D, Sánchez-Lozada LG, Kang DH, Ritz E. Uric acid and chronic kidney disease: which is chasing which? Nephrol Dial Transplant. 2013;28(9):2221–28.