Indian Journal of Experimental Biology
Vol. 63, September 2025, pp. 746-754
DOI: 10.56042/ijeb.v63i09.18893

Amelioration of kidney damage in diabetic rat by *Catharanthus roseus* (L.) G. Don extract

Abhishek Byahut[1], Arundhati Bag[1]*, Chamma Gupta[1] & Mingma Sherpa[2]

[1]Department of Medical Biotechnology, [2]Department of Pathology, Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, Gangtok, Sikkim 737102, India

Received 25 April 2025; revised 18 May 2025

Catharanthus roseus (L). G. Don is used in traditional medicine worldwide to treat various diseases including diabetes. Present study was carried out to evaluate the protective effect of aqueous leaf extract of *C. roseus* (CR) on type 2 diabetes mellitus (T2DM)- associated nephropathy in a rat model. Streptozotocin and nicotinamide-induced T2DM Wistar rats were fed with CR extract (200, 400 and 600 mg/kg body weight) daily, for eight weeks. Fasting blood glucose (FBG) level was monitored at regular intervals. Urine and serum biochemical markers were measured after sacrificing the animals at the end of eight weeks of treatment. Kidney tissues were used for histopathological examinations and for western blot analysis of nephrin, a slit diaphragm protein of glomerular filtration barrier. The results showed a dose-dependent decrease in FBG in CR-treated diabetic animals. CR treatment led to normalisation of serum lipid markers, and urine albumin-creatinine ratio which were elevated in diabetic groups. Histopathological analysis demonstrated a reduction of glomerular abnormalities in diabetic rats after CR-treatment. Further, CR doses of 200 mg and 400 mg/kg body weight significantly improved nephrin expression in kidney. CR was highly rich in alkaloids and flavonoids along with other phytochemical compounds as found in qualitative phytochemical analysis. CR protected kidneys from high sugar-induced damage in diabetic rats. While phytochemicals of *C. roseus* are well known for their anti-diabetic properties, they are yet to be explored in depth as potential therapeutic agent(s) for diabetic kidney disease.

Keywords: Diabetes, Diabetic nephropathy, Nephrin, Phytochemicals

The rising curve of diabetes mellitus (DM) has become a major public health concern in both developed and developing countries. According to the International Diabetes Federation (2025)[1], about 590 million people worldwide are having diabetes, and by 2050 the number will increase by 46%. A persistent glucose exposure causes macro- and microvascular complications in DM. Diabetic nephropathy (DN), a microvascular complication of DM, is characterised by the presence of abnormal levels of proteins in urine and may lead to progressive decline in kidney function and enhanced cardiovascular problems. DN is a single major cause of end-stage renal disease (ESRD). Around 20-40% of type 1 and type 2 diabetes mellitus patients can develop DN[2], which increases morbidity and mortality in diabetic individuals[3]. At the initial stage of nephropathy or microalbuminuria, there is a low but persistent presence of abnormal levels of albumin in urine, i.e., 30–299 mg/24 h. Whereas in overt nephropathy or macroalbuminuria, albumin level in urine is ≥ 300 mg/24 h persistently. In type 1 diabetes overt nephropathy may develop after many years but it may be present in individuals with type 2 diabetes at the time of diagnosis. Overt nephropathy usually progresses to ESRD[4]. In a study on three decades-long diabetic incidences worldwide, it was found that T2DM accounted for the major burden of diabetic kidney disease[5].

A disruption of glomerular filtration barrier (GFB) precedes diabetic nephropathy. Podocytes constitute the outermost layers of GFB, and work with the glomerular basement membrane and innermost fenestrated endothelial layer to ensure efficient filtration in the kidneys. Podocyte foot processes (FP) form slit diaphragm which acts as sieve and prevents the loss of essential proteins in the urine. Hyperglycemia causes injury in podocytes and effacement of its foot processes resulting in leakage of albumins in urine[6,7]. Nephrin is a transmembrane protein of podocyte, which is a major component of podocyte FP and slit diaphragm. Hyperglycaemia induces down-regulation of nephrin

*Correspondence:
E-mail: arundhatis5@rediffmail.com

in podocyte[8] that affects the integrity of slit diaphragms.

Intensive treatments controlling blood glucose and blood pressure reduce diabetic complications. Despite significant advancements in diabetes management, many people with DN still require dialysis or renal transplantation, and approximately 10% of deaths in T2DM occur due to renal failure[9]. Further, long term use of the drugs can cause adverse effects and it is also difficult to bear the cost of these drugs for people with low annual income. Therefore, there is scope to develop new alternative drugs for DN. Plants constitute rich source of phytochemicals with medicinal properties for several diseases. Plant-based management of diabetes and diabetic complications are an age-old practice in traditional medicine. Plant-based medicine, which was more common in rural areas nowadays is becoming popular in urban areas also for its fewer side effects in comparison to the synthetic drugs.

C. roseus, also known as *Vinca rosea,* periwinkle, sadabahar, nayantara and taraphool, is an herbaceous, perennial plant with height and width ranging from 7 to 24 inches. It belongs to family Apocynaceae. Native to Madagascar, it is cosmopolitan in distribution and is widely distributed in India. It is an ornamental plant and has gained much attention for its potential pharmacological effects in treating various diseases including cancer, hypertension, neural inflammation, and diabetes[10]. However, its effectiveness on diabetic kidney remains largely unexplored. Therefore, in the present study an experimental attempt was made to evaluate the protective effect of *C. roseus* leaf extract on T2DM in Wistar rats.

Materials and Methods

Plant collection and identification

Fresh leaves of *C. roseus* was collected from the village of Kitam, South Sikkim (India) in the month of April 2021. The plant was identified and authenticated by taxonomist in the Department of Botany, Sikkim University. The voucher specimen (Herbarium Accession Number 639) was also submitted to the department. For improved preservation of temperature and light-sensitive molecules, collected plant specimens were cleaned, washed, and allowed to air dry for a few days at room temperature in a dark, well-ventilated area, and then powdered using a mixer grinder.

Preparation of plant extracts

Aqueous extract of *C. roseus* leaves was prepared in conical flask containing 10 g of dry plant powder macerated in 100 mL of distilled water for 72 hours and the macerated mixture was kept on rotatory shaker for 3 hours. After that, it was filtered using Whatman filter paper #1 to get rid of solid particles. The supernatant was collected, and the samples were lyophilized after freezing over night by using freeze dryer method. They were stored at -20 °C until further use.

Qualitative phytochemical analysis

The aqueous extract of *C. roseus* was screened for the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, terpenoids and tannins using various biochemical methods described by Harborne[11]. In general, Wagner's reagent was used to confirm the presence of alkaloids, lead acetate test for flavonoids, ferric chloride test for phenols, foam test for saponins, Salkowski test for terpenoids and potassium dichromate test for tannins.

Animal handling and ethics statement

The experiment was carried out on male Wistar rats weighing 120-180g, and aged 7-9 weeks which were purchased from CPCSEA registered vendor (1443/PO/Br/s/11/CPCSEA). Males were preferred as female rats can show more hormonal fluctuations during reproductive cycles. It is also found that oestrogen can increase insulin sensitivity in female diabetic rats[12]. The rats were kept in stainless steel cages under controlled conditions in a lab environment (24 ±2 °C, relative humidity 45–55 %, and 12-hour light/dark cycle). Water *ad libitum*, the animals were fed with commercial rat food. All experiments were performed following relevant regulatory standards. The study protocol was approved by the Institute's Animal Ethics Committee (Reference no: MC/SMIMS/IAEC/01/2019 dated 6.8.2019).

Induction of T2DM

The rats were acclimatised for one week and fasted for an overnight period before the experiment, with unrestricted access to water. To induce T2DM in the animals, a single dose of 65 mg/kg bodyweight (bw) streptozotocin (STZ) (SRL, India) freshly made in 0.1 M citrate buffer (pH 4.5) was administered intraperitoneally (IP) 15 minutes after an IP injection of nicotinamide NA (SRL, India) (110 mg/kg bw dissolved in normal saline). The animals were then

provided with 5% sucrose for 24 hours to prevent fatal hypoglycaemia. The animals were monitored, and following overnight fast, fasting blood glucose (FBS) was measured by glucometer (Accu-Chek® Active, Roche, Germany) from the tail vein blood 72 hours after STZ administration. Diabetic rats with FBS levels greater than 200 mg/dL were included in the study[13,14].

Animal grouping and treatment

The rats were kept in standard laboratory conditions throughout the study. Diabetes was induced in rats a week before the experimentation. They were parted into different groups after the induction of diabetes. The rats were administered with various concentrations of crude extract of *C. roseus* via oral gavage once daily for eight weeks. Normal control and diabetic control were treated with vehicle (distilled water) only over the same treatment period. The selection of *Catharanthus roseus* (CR) doses was based on the therapeutic ranges as found in published literature[15,16]. In addition to this, literature on toxicity was also searched. When aqueous leaf extract of CR was administered orally in both acute and sub-acute toxicity studies in male Wistar rats, no mortality and significant toxicity was observed, even at doses as high as 10,000 mg/kg[17].

The animals were divided into 6 groups with 6 rats per group: Diabetic rats treated with 200 mg aqueous extract /kg bw (CR-200); Diabetic rats treated with 400 mg aqueous extract /kg bw (CR-400); Diabetic rats treated with 600 mg aqueous extract /kg bw (CR-600); Untreated normal control (NC) rats; Untreated diabetic control (DC) rats and Diabetic rats treated with 5 mg Glibenclamide /kg bw (GBC-5).

Changes in body weight and estimation of blood glucose

Body weights (bw) of rats were measured on 0 day and at the end of every week of the treatment, continuing till the end of treatment duration. Blood samples were collected from the tail vein, and FBS was measured by glucometer in all the rats every week at the end of the treatment.

Urine sample collection

Urine samples of the rats were collected over 24-hour periods at the end of 8th week treatment duration by housing the individual rat in isolated cages by polyethylene funnel method[18]. Urine albumin and creatinine were measured by commercially available kits (Transasia, ERBA).

Collection of kidney and blood samples

At the end of the eight-week post-treatment, the rats were anaesthetized with diethyl ether and blood samples were collected by cardiac puncture. Blood was centrifuged at 4000 rpm for 10 minutes to obtain the serum and was stored at -40 °C until further investigations. The rats were sacrificed by cervical dislocation and the kidneys were dissected, washed with chilled 0.9% NaCl and cleaned of surrounding tissues. Finally, a part of kidney section from each group was collected for western blot analysis and stored in -80°C till further study. The other part of kidney was fixed in phosphate-buffered saline (PBS) containing 10% formalin for histopathology.

Biochemical analysis

Serum biochemical parameters like total protein, albumin, globulin, HbA1c, HDL and LDL cholesterols, triglycerides, were assessed following the protocol instructed by the manufacturer (Transasia, ERBA).

Histological assessment of the kidney

The kidneys were fixed in 10% formalin for 48 hours (post-fixation). Paraffin blocks were prepared using standard histological techniques. The embedded tissue sections were taken for a thickness of 5μm, stained with hematoxylin and eosin (Sigma-Aldrich), and examined under 10× and 40× of a light microscope (Leica, Hamburg, Germany).

Western blot analysis

Proteins were extracted from renal tissues using RIPA solution and the supernatant was obtained after centrifuging at 12,000*g* at 4°C for 30 minutes. The protein concentration was detected using the assay kit for BCA protein (Thermo Fisher Scientific, catalog # PA5-79756). Total protein was separated by 8% SDS-PAGE and transferred onto nitrocellulose membrane. After blocking in PBST containing 5% skim milk for 1 hour at room temperature, the membranes were incubated for overnight at 4 °C with primary antibody (anti-Nephrin antibody 1:2000; Thermo Fisher Scientific, catalog # 23227, and GAPDH antibody Thermo Fisher Scientific, catalog # PA1-987). The membranes were subsequently incubated with the appropriate HRP-conjugated secondary antibody for 2 hours at room temperature (Thermo Fisher Scientific, catalog A18921), and protein bands were detected by electrochemiluminescence (ECL). Image lab software (Bio-Rad, Hercules, CA, USA) was used

to analyse the protein band intensities. This part of work was performed at National Institute of Biomedical Genomics Kalyani, West Bengal.

Statistical analysis

The GraphPad Prism software package, version 9.5.1, was used to analyse the results. The mean ± standard error of the mean (SEM) was computed for continuous parameters. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for the effect of different doses of CR was used to compare biochemical parameters within groups. Values were statistically significant at P - value < 0.05

Results

Qualitative phytochemical analysis

After the completion of the extraction process, a total of 13.75% by weight of dry crude aqueous extract of *C. roseus* was obtained. The extract exhibited a rich phytochemical profile. The alkaloids, flavonoids were very high followed by terpenoids and tannins. Additionally, phenols and saponins showed positive responses, albeit in low concentrations compared to the other phytochemicals.

Analyses of blood glucose and body weight in experimental animals

When comparing the diabetic rats to the normal control group, a significant rise in fasting blood glucose was observed (Fig. 1A); this difference became more pronounced at the 8th week mark after the induction of diabetes. Additionally, by the eighth week, the administration of CR 200, 400, and 600 mg/kg bw to diabetic rats resulted in a dose-dependent decrease in blood sugar levels by 32.6%, 41.3%, and 49.3%, respectively, when compared to the blood glucose in first week of diabetes.

Fig. 1B depicts body weight changes for different treatments. Except the normal control, all other groups showed decline in body weight till the 4th week of study. Thereafter it was increased for all the treated groups till the end of the study, although the graphs were below baseline. Body weight of normal control group increased significantly (13.60%) by 8th week. The diabetic control group, on the other hand, experienced weight loss throughout the study with 34.67% decrease at the end of 8th week. The treatment groups also had reductions in body weight by 17.49%, 21.48% and 18.60% however it was less than the diabetic control group.

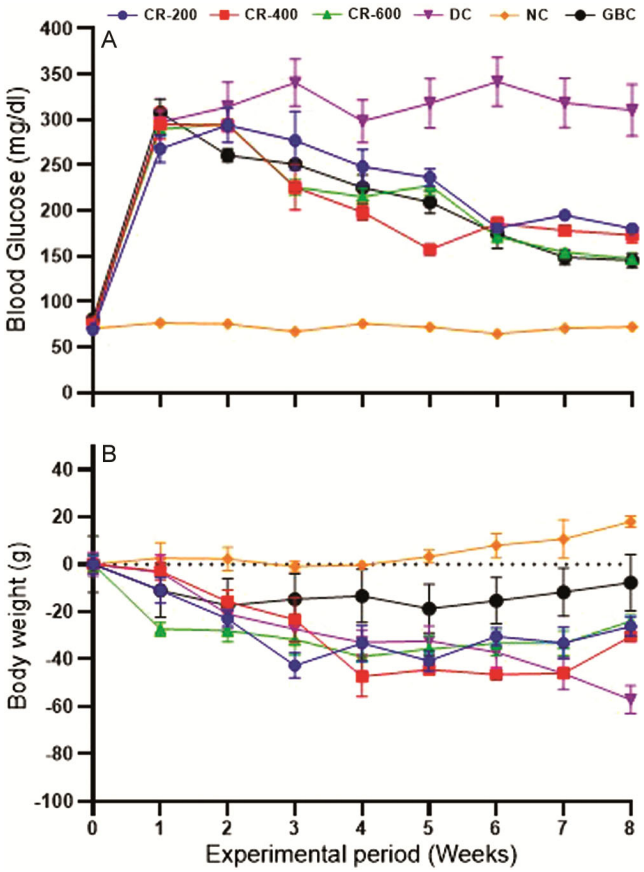

Fig. 1 — Graphical presentation of the effects of different treatments of *C. roseus* on (A) fasting blood glucose and (B) body weight. [Data represented as mean ± SEM (n=6). CR: diabetic rats treated with *C. roseus,* DC: diabetic control treated with vehicle, NC: normal control treated with vehicle; GBC: diabetic rats treated with glibenclamide]

Analyses of biochemical parameters

Fig. 2 demonstrates changes in biochemical parameters in different groups. Percentage of glycated haemoglobin (HbA1c %) was found to be significantly (P < 0.001) higher in the untreated diabetic control group (10.03± 4.31) than in the normal control group (1.40 ± 0.47). During the investigation, HbA1c was significantly (P < 0.001) normalised after treatment with all three dosages of CR extract in comparison to the diabetic control group (4.37±0.45, 1.20±0.41, and 1.23± 0.14, for CR-200, CR-400 and CR-600, respectively), indicating the anti-hyperglycaemic effects of the *C. roseus*. Additionally, there was a decrease in serum albumin and total protein levels in diabetic rats. However, treatment with different doses of CR extracts led to the normalisation of these parameters. In diabetic control rats, there was a significant (P < 0.01)

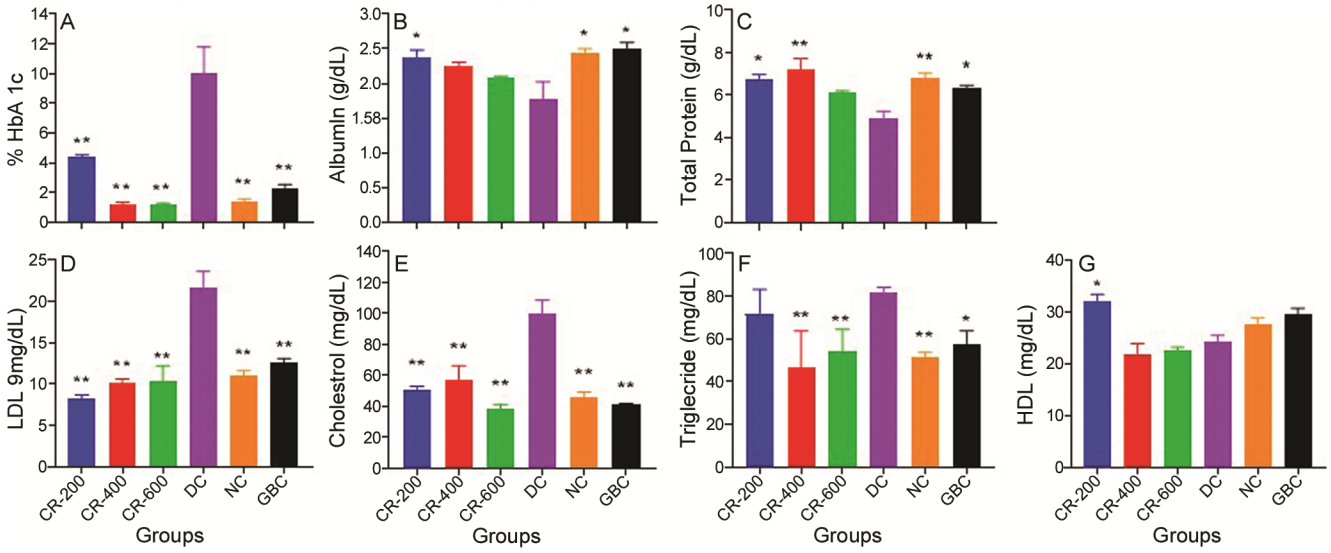

Fig. 2 — Effect of *C. roseus* extract on biochemical parameters in STZ-NA-induced diabetic rats. {Data expressed as mean ± SEM. One-way ANOVA followed by Tukey's multiple comparisons test. Compared to diabetic control group: *$P < 0.05$, ** $P < 0.001$]

increase in total cholesterol, TG and LDL cholesterol compared to normal control. In addition, there was a decrease in HDL cholesterol in diabetic control rats in comparison to normal control. Administration of the crude aqueous extract (CR-200, CR-400 and CR-600 mg/kg bw) for eight weeks showed a significant ($P < 0.01$) reduction in total cholesterol, TGs and LDL cholesterol in comparison to the diabetic control rats. The effect was comparable to the standard drug glibenclamide (5mg/kg bw).

There were significant differences in albumin/creatinine ratio (ACR) in urine (Fig. 3) between the control group (21.35± 3.93 mg/dL) and in the diabetic group (101.12 ±5.11 mg/dL). The treatment groups CR-200, CR-400 and CR-600 showed significantly lower ACR value, i.e., 40.30±1.36, 42.66±3.9 and 35.28± 2.39 respectively, when compared with the diabetic control group.

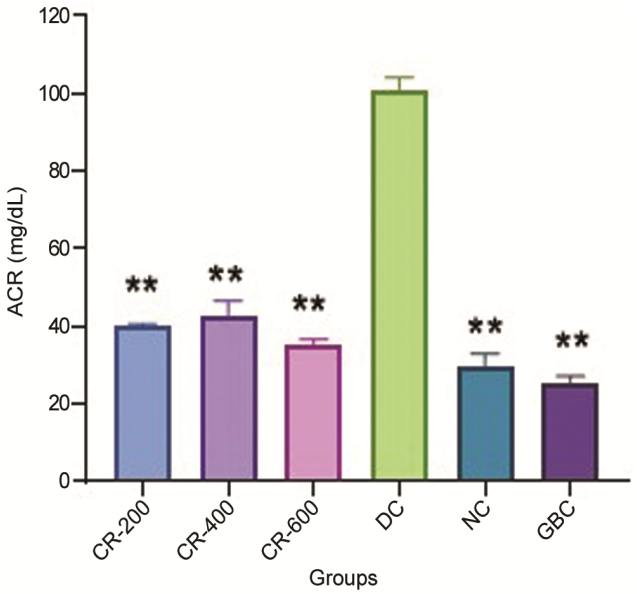

Fig. 3 — Effect of long-term treatment of *C. roseus* leaf extracts on urinary albumin/creatinine ratio (ACR) in diabetic rats. [Values are mean ± standard deviation. **$P < 0.001$]

Histological examination of kidney tissue damage in diabetic rats

Effect of CR treatments on hyperglycaemia-mediated kidney tissue damage was observed through histological studies in the different groups of rats. Thickening of the kidney arterial wall, rise in the Bowman's space in kidney glomerular areas were observed in the diabetic control group. Treatment with CR-200, CR-400 and CR-600 showed reduction in pathological alterations and lowered hyperglycaemia-mediated kidney damage in diabetic animals (Fig. 4).

Effect of *Catharanthus roseus* on nephrin

Western blot studies showed the level of nephrin in different groups (Fig. 5). Renal nephrin level was less in diabetic control rats. Eight weeks of CR treatments with 200 and 400 mg/kg bw significantly elevated the nephrin level in the kidney of diabetic rats and glibenclamide treatment (5mg/kg bw) partially improved level of glomerular nephrin.

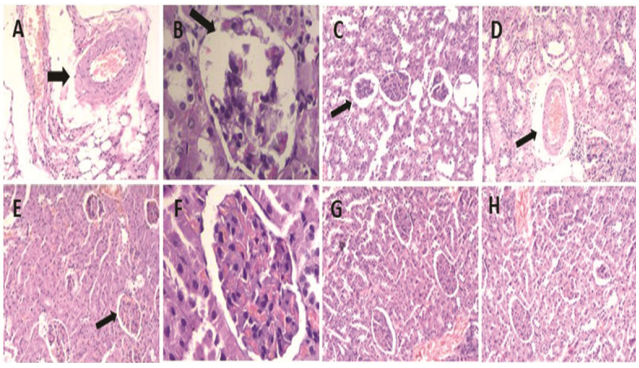

Fig. 4 — Histopathological changes in kidney of normal control, diabetic control, treated groups (CR 200, 400, 600 mg/kg) of rats. H&E-stained kidney tissue sections from (A), (B) & (C) diabetic control (thickening of the renal arterial wall in the diabetic kidney and increase in bowman's space of kidney), (D) & (E) normal control (represents normal renal arterial wall with normal glomerulus), (F) CR 200 mg/kg treated diabetic, (G) CR 400 mg/kg treated diabetic, (H) CR 600 mg/kg treated diabetic rats.

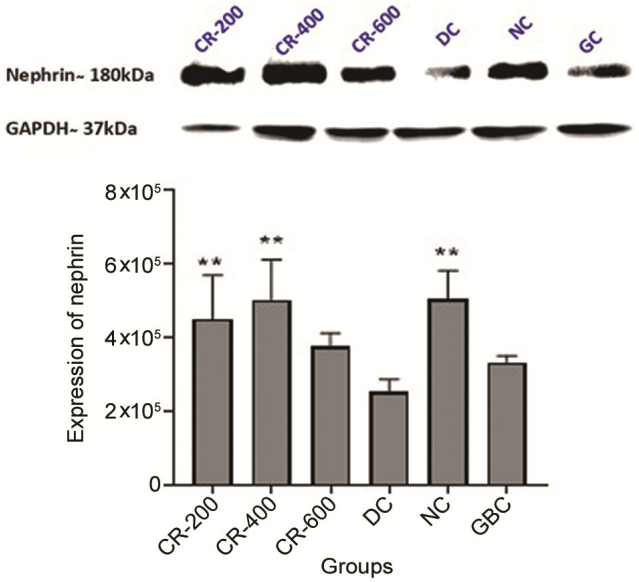

Fig. 5 — Western blot analysis and a summary graph for the densitometry values of the nephrin signal blotted from the kidney lysates of the control and treatment groups of rats. GAPDH used as loading control. [Values are mean ± standard deviation. $^{**}P < 0.01$]

Discussion

Present day management for diabetic nephropathy includes control of blood glucose, blood pressure and lipid along with the modification of lifestyle factors. The renin-angiotensin system inhibitor and sodium glucose cotransporter 2 inhibitor are widely used treatment options[19]. Despite all these developments, diabetic nephropathy continues to be a significant complication of T2DM.

The present study investigates reno-protective effects of *C. roseous* aqueous leaf extract in diabetic rat model. In the untreated diabetic rats, chronic hyperglycemia increased glycated hemoglobin, cholesterol, LDL, and triglycerides. Dyslipidemia, particularly in type 2 diabetes, is linked to DN in addition to impaired glucose metabolism[20]. High urine albumin/creatinine ratio was observed in comparison to the normal control group. When CR extracts were administered to the diabetic rats, these measures were reduced. Also, there was significant weight loss in diabetic animals, which was improved following treatment with *C roseus* extracts. Although all CR-treated groups showed a trend toward weight gain compared to the diabetic control, they still exhibited lower body weights than the normal control group**.** This potentially suggests partial, but not complete, metabolic recovery**.** Weight loss in diabetes is commonly associated with poor glucose utilisation, muscle wasting, and fat catabolism. The CR extract may have improved glycaemia parameters and renal function, but its impact on overall metabolic health might be limited at the studied duration or dose range. Alternatively, this pattern could suggest that while CR mitigated organ-specific damage (like nephropathy), it did not fully reverse systemic metabolic imbalance, pointing to the need for combination therapy or longer treatment periods.

CR treated groups showed reduction in the pathological changes and had improved kidney architecture. Western blot data demonstrated low renal nephrin levels in diabetic control rats. As mentioned earlier, nephrin is an integral part of podocyte foot processes. Thus, its reduced level in western blot is a potential indicator of kidney damage. It is established that hyperglycaemia reduces nephrin expression and results in podocyte depletion[8]. The results of our study demonstrated that CR treatment significantly elevated the nephrin level in kidney of diabetic rats. Interestingly, however, the highest dose group (CR-600) did not show significant improvement in nephrin expression compared to the diabetic control, unlike the CR-200 and CR-400 groups. This may indicate a dose-dependent biphasic effect, where lower to moderate doses exhibit therapeutic benefits, while higher doses may lead to diminished efficacy or potential toxicity[21]. Similar trend was observed in previous studies, where phytochemical overload at high concentrations potentially exerted oxidative or cytotoxic stress, offsetting the expected reno- protective effects[22].

Qualitative phytochemical study showed that *C. roseus* extract was highly rich in flavonoids and alkaloids. Flavonoids are important phytochemicals mostly having medicinal values. These compounds have potential therapeutic benefits for various diseases including diabetes and its related complications, such as nephropathy[23]. Flavonoids, for example, can protect diabetic kidneys in multiple pathways especially by reducing oxidative stress and inflammation[24]. Some of the flavonoids also reduce lipid peroxidation, upregulate antioxidant enzymes and their activities[25]. The protective effects observed with *C. roseus* (CR) extract, particularly at 200 and 400 mg/kg can be attributed to its rich content of bioactive alkaloids and flavonoids. Its mechanism likely involves reduction of oxidative stress, which is central to the pathogenesis of diabetic nephropathy. Vindoline and other CR constituents may attenuate reactive oxygen species (ROS) generation, enhance endogenous antioxidant defence systems (e.g., SOD, CAT, GPx), and inhibit AGE (advanced glycation end product) accumulation, thereby preserving nephrin expression and podocyte integrity[26]. Additionally, flavonoids present in CR may modulate inflammatory and fibrotic pathways, particularly through NF-κB inhibition and downregulation of *TGF-β1*, both of which are critical drivers of glomerulosclerosis. These findings align with earlier reports indicating that CR-derived compounds act through multiple reno-protective signalling cascades, contributing to functional and structural improvement in diabetic kidneys[26-28].

C. roseus is well known for its medicinal value, especially for cancer and diabetes. However, it is very rarely studied for its protective role in diabetic kidney. Only one study by Oguntibeju *et al.*[26] demonstrated the protective effect of vindoline alkaloid *C. roseus* on kidney pathophysiology in diabetic rats. No other study was found in PubMed, demonstrating reno- protective effect of this plant. We studied *C. roseus* crude extract in T2DM rats with DN. The crude extract contains various phytochemicals, which may function in different ways and can act synergistically. Therefore, they may be more effective in combination than as single agent[29]. While the study demonstrates the therapeutic potential of *C. roseus* extract in diabetic nephropathy, it lacks evaluation of individual bioactive constituents such as vindoline or serpentine. The absence of isolated compound testing limits our ability to attribute the observed effects to specific phytochemicals. Moreover, pharmacokinetic profiling of the extract was not conducted, leaving gaps in understanding the absorption, bioavailability, metabolism, and tissue distribution of active components. Future studies incorporating LC-MS-based phytochemical quantification, compound isolation, and pharmacokinetic analysis are necessary to validate and optimize therapeutic use. Findings of this study could pave the way to identify more than one potentially effective molecule for kidney protection in hyperglycemia. This study can be further extended for isolation and identification of specific phytochemical(s) in *C. roseus* leaf extract responsible for the kidney-protecting properties. Further, their toxicity profile should be carefully analysed because molecules from natural origins are not always harmless. For example, vinca alkaloids are often associated with neurotoxicity[30]. To evaluate the translational relevance of the findings, the doses of *C. roseus* extract used in rats (200–600 mg/kg) can be converted to human-equivalent doses (HED) using standard allometric scaling. Applying the FDA-recommended conversion factor (rat-to-human = 6.2)[31], the highest dose (600 mg/kg) in rats equates to approximately 97 mg/kg in humans, or ~6.8 g/day for a 70 kg adult. While this suggests potential feasibility, clinical translation must consider differences in human metabolism, extract standardisation, and safety profiles. Further clinical studies are required to determine optimal dosing, efficacy, and toxicity in humans. Altogether, it can be said that the present study sheds light on the potential protective effect of *C. roseus* extract on diabetic kidney.

Conclusion

Little information is available on the protective effect of *C. roseus* on a diabetic kidney. The present study shows that *C. roseus* could be a potential source of therapeutic agent(s) for the management of diabetic nephropathy. This investigation demonstrated the protective effects of *C. roseus* on nephrin, a slit diaphragm protein of podocytes in a rat model of diabetic nephropathy. Further study identifying the most effective compound(s) would contribute to developing an effective drug for diabetic nephropathy.

Acknowledgements

Department of Biochemistry, Microbiology, and Pathology, Sikkim Manipal Institute of Medical sciences, Sikkim Manipal University are specially acknowledged for providing laboratory facilities.

Authors sincerely thank Dr. Anupam Basu for providing laboratory support in National Institute of Biomedical Genomics, Kalyani, and West Bengal.

Funding statement

Authors thankfully acknowledge the financial support from Himalayan Fellowship, National Mission on Himalayan Studies, Govt. of India, Grant Number GBPNI/NMHS-2018-19/HSF23-01/152, Ministry of Environment, Forest and Climate Change, Govt. of India.

Conflict of interest

The authors declare no conflicts of interest.

References

1 International Diabetes Federation, IDF diabetes atlas. *Int Diabetes Fed*, (2025) 1. (Accessed on 18.04.2025).

2 Persson F & Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney Int Suppl* (*2011*), 8 (2018) 2. https://doi.org/10.1016/j.kisu.2017.10.003

3 Dounousi E, Duni A, Leivaditis K, Vaios V, Eleftheriadis T & Liakopoulos V. Improvements in the management of diabetic nephropathy. *Rev Diabet Stud*, 12 (2015) 119. https://doi.org/10.1900/RDS.2015.12.119

4 Lim Akh. Diabetic nephropathy - complications and treatment. *Int J Nephrol Renovasc Dis*, 15 (2014) 361. https://doi.org/10.2147/IJNRD.S40172

5 Deng Y, Li N, Wu Y, Wang M, Yang S, Zheng Y, Deng X, Xiang D, Zhu Y, Xu P, Zhai Z, Zhang D, Dai Z & Gao J. Global, regional, and national burden of diabetes-related chronic kidney disease from 1990 to 2019. *Front Endocrinol* (*Lausanne*), 12 (2021) 672350. https://doi.org/10.3389/fendo.2021.672350

6 Tao Y, Chaudhari S, Shotorbani PY, Ding Y, Chen Z, Kasetti R, Zode G & Ma R. Enhanced Orai1-mediated store-operated Ca^{2+} channel/calpain signaling contributes to high glucose-induced podocyte injury. *J Biol Chem*, 298 (2022) 101990. https://doi.org/10.1016/j.jbc.2022.101990

7 Barutta F, Bellini S & Gruden G. Mechanisms of podocyte injury and implications for diabetic nephropathy. *Clin Sci* (*Lond*), 136 (2022) 493. https://doi.org/10.1042/CS20210625

8 Wu T, Ding L, Andoh V, Zhang J & Chen L. The mechanism of hyperglycemia- induced renal cell injury in diabetic nephropathy disease: an update. *Life* (*Basel*), 13 (2023) 539. https://doi.org/10.3390/life13020539

9 Jitraknatee J, Ruengorn C & Nochaiwong S. Prevalence and risk factors of chronic kidney disease among type 2 diabetes patients: A cross-sectional study in primary care practice. *Sci Rep*, 10 (2020) 6205. https://doi.org/10.1038/s41598-020-63443-4

10 Almagro L, Fernández-Pérez F & Pedreño MA. Indole alkaloids from *Catharanthus roseus*: bioproduction and their effect on human health. *Molecules*, 20 (2015) 2973. https://doi.org/10.3390/molecules20022973

11 Harborne JB. *Phytochemical Methods: A guide to modern techniques of plant analysis.* (1973) Chapman & Hall Ltd., London, UK.

12 Díaz A, López Grueso R, Gambini J, Monleón D, Mas Bargues C, Abdelaziz KM, Viña J & Borrás C. Sex differences in age-associated type 2 diabetes in rats-role of estrogens and oxidative stress. *Oxid Med Cell Longev*, 2019 (2019) 6734836. https://doi.org/10.1155/2019/6734836

13 Nayak Y, Hillemane V, Daroji VK, Jayashree BS & Unnikrishnan MK. Antidiabetic activity of benzopyrone analogues in nicotinamide-streptozotocin induced type 2 diabetes in rats. *Sci World J*, 2014 (2014) 854267. https://doi.org/10.1155/2014/854267

14 Qinna NA & Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. *Drug Des Devel Ther*, 9 (2015) 2515. https://doi.org/10.2147/DDDT.S79885

15 Alqahtani AS, Ullah R & Shahat AA. Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia. *Evid Based Complement Alternat Med*, 17 (2022):7123521. https://doi:10.1155/2022/7123521.

16 Al-Shaqha WM, Khan M, Salam N, Azzi A & Chaudhary AA. Anti-diabetic potential of *Catharanthus roseus* Linn and its effect on the glucose transport gene (GLUT-2 and GLUT-4) in streptozotocin induced diabetic wistar rats. *BMC Complement Altern Med*, 15 (2015) 379. https://doi:10.1186/s12906-015-0899-6.

17 Ukoha AI, Okereke SC, Arunsi UO, Ngwogu AC, Jack AB, Chukwudoruo SC & Bello HA. Sub-lethal assessment of aqueous dried leaf extracts of *Catharanthus roseus* (Linn.) G. Don in male albino rats. *MOJ Toxicol*, 3 (2017) 128. https://doi.org/10.15406/mojt.2017.03.00068

18 Kurien BT, Everds NE & Scofield RH. Experimental animal urine collection: a review. *Lab Anim*, 38 (2004) 333. https://doi.org/10.1258/0023677041958945

19 Yamazaki T, Mimura I, Tanaka T & Nangaku M. Treatment of diabetic kidney disease: Current and future. *Diabetes Metab J*, 45 (2021) 11. https://doi.org/10.4093/dmj.2020.0217

20 Kaur N, Kishore L & Singh R. *Dillenia indica* L. attenuates diabetic nephropathy via inhibition of advanced glycation end products accumulation in STZ-nicotinamide induced diabetic rats. *J Tradit Complement Med*, 8 (2017) 226. https://doi.org/10.1016/j.jtcme.2017.06.004

21 Jodynis-Liebert J & Kujawska M. Biphasic dose-response induced by phytochemicals: Experimental evidence. *J Clin Med*, 9 (2020):718. https://doi:10.3390/jcm9030718.

22 Vutukuri VR, Das MC, Reddy M, Prabodh S & Sunethri P. Evaluation of acute oral toxicity of ethanol leaves extract of *Catharanthus roseus* in Wistar albino rats. *J Clin Diagn Res*, 11 (2017) FF01 DOI: 10.7860/JCDR/2017/24937.9325.

23 Vargas F, Romecín P, García Guillén AI, Wangesteen R, Vargas Tendero P, Paredes MD, Atucha NM & García Estañ J. Flavonoids in kidney health and disease. *Front Physiol*, 9 (2018) 394. https://doi.org/10.3389/fphys.2018.00394

24 Hu Q, Qu C, Xiao X, Zhang W, Jiang Y & Wu Z. Flavonoids on diabetic nephropathy: advances and therapeutic opportunities. *Chin Med*, 16 (2021) 74. https://doi.org/10.1186/s13020-021-00485-4

25 Yi X, Dong M, Guo N, Tian J, Lei P, Wang S, Yang Y & Shi Y. Flavonoids improve type 2 diabetes mellitus and its complications: a review. *Front Nutr*, (2023) 1192131. https://doi.org/10.3389/fnut.2023.1192131

26 Oguntibeju OO, Aboua Y & Goboza M. Vindoline-A natural product from *Catharanthus roseus* reduces hyperlipidemia and renal pathophysiology in experimental type 2 diabetes. *Biomedicines*, 7 (2019) 59. https://doi.org/10.3390/biomedicines7030059

27 Choy KW, Murugan D, Leong XF, Abas R, Alias A & Mustafa MR. Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B (NFκB) signaling in cardiovascular diseases: A mini review. *Front Pharmacol*, 10 (2019) 1295 DOI: 10.3389/fphar.2019.01295.

28 Alsawaf S, Alnuaimi F, Afzal S, Thomas RM, Chelakkot AL, Ramadan WS, Hodeify R, Matar R, Merheb M, Siddiqui SS & Vazhappilly CG.Plant flavonoids on oxidative stress-mediated kidney inflammation. *Biology*, 11 (2022) 1717 DOI: 10.3390/biology11121717.

29 Fisher DR, Zheng T, Bielinski DF, Kelly ME, Cahoon DS & Shukitt Hale B. Phytochemical combination is more effective than individual components in reducing stress signaling in rat hippocampal neurons and microglia in vitro. *Int J Mol Sci*, 23 (2022) 12651. https://doi.org/10.3390/ijms232012651

30 Moudi M, Go R, Yien CYS & Nazre M. Vinca alkaloids. *Int J Prev Med*, 4 (2013) 1231.

31 US Food and Drug Administration (FDA). *Guidance for Industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers.* Center for Drug Evaluation and Research (CDER), Rockville, MD; 2005.