OPEN ACCESS

Saudi Journal of Biomedical Research

Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Investigating the Renoprotective Effect of Nintedanib against Diabetic Nephropathy on Albino Wistar Rats

Sabarinath Chandrasekar[1]*, Monika Moorthi[1], Vaishnavi Kumaresan[1], Sudhakar Pachiappan[1], Poorana Pushkalai Saravanan[1]

[1]Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Tamilnadu – 637205, India

DOI: https://doi.org/10.36348/sjbr.2025.v10i03.001 | **Received:** 23.01.2025 | **Accepted:** 28.02.2025 | **Published:** 06.03.2025

***Corresponding author:** Sabarinath Chandrasekar
Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Tamilnadu – 637205, India

Abstract

Aim of current study is to perform and investigate the renoprotective effect of nintedanib on diabetic nephropathy in albino wistar rat. Diabetic nephropathy was induced in overnight fasted rats by a single intraperitoneal injection of Streptozotocin (STZ) (45 mg/kg) prepared freshly in ice chilled 0.1 M citrate buffer (Ph-4.5) and after 15 minutes Nicotinamide (110 mg/kg)in in 0.9% normal saline was administered. The rat were divided into five grouping six animal in each group Each group underwent a different treatment protocol Treatment with nintedanib at 30 mg/kg and 50 mg/kg was evaluated using invivo studies such as physiological evaluation, serum and urine biochemical parameters like creatinine, albumin, BUN, uric acid and urine albumin level and histological studies of diabetic nephropathy induced rat's kidney. The present study demonstrated that the treatment of nintedanib exerts its renoprotective potential against the progression of diabetic nephropathy. The results showed significant improvements in renal function parameters and reduced histological changes when compared to the disease control group. Thereby, nintedanib at 50 mg/kg has demonstrated better effectiveness than nintedanib at 30 mg/kg. Our finding suggests that nintedanib holds potential as a novel therapeutic agent in the management of diabetic nephropathy by inhibiting TGF-L. vascular endothelial growth factors, and their signaling pathways in the progression of diabetic nephropathy. Further research is needed to elucidate its long-term safety and efficacy in clinical treatments.

Keywords: Diabetic Nephropathy, Renoprotective Effect, Nintedanib, Tyrosine kinase inhibitor.

1. INTRODUCTION

High blood glucose levels caused by absolute or relative insulin insufficiency along with beta-cell malfunction and insulin resistance are the hallmarks of diabetes mellitus (DM), a chronic metabolic disease [1, 2]. It also causes aberrant insulin production, insulin receptor, or post-receptor processes that alter metabolism and involve the liver, kidney, and pancreatic β cells [3, 4].

Diabetic nephropathy (DN) is a microvascular complication that is one of the main causes of mortality linked with diabetes mellitus [5]. Both type I and type II diabetes can result in diabetic nephropathy, and DN is a major cause of renal dysfunction in affluent nations [6, 7].

With the onset of glomerular hypertension, DN results in several alterations to the renal structures (arteries, arterioles, kidney glomeruli, and tubules), which frequently leads to the development of glomerulosclerosis and chronic renal failure (CRF) [8]. Microalbuminuria screening should generally be done every year, beginning five years following the diagnosis of type I and type II diabetes [9, 10].

Molecular mechanisms that involved in diabetic nephropathy, includes increase in production of advanced glycation end products (AGE) activates the growth factor-I, transforming growth factor beta (TGF β), vascular endothelial growth factor (VEGF) in mesangial cells which in turn produce collagen type IV [11].

Nintedanib, a tyrosine kinase inhibitor approved for the treatment of idiopathic pulmonary fibrosis, has anti-fibrotic, anti- inflammatory, and anti-angiogenic activity [12].

Nintedanib is a well-known broad-spectrum tyrosine kinase inhibitor that targets multiple receptor

kinases, such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast derived growth factor (FDGF) and also blocks transforming growth factor beta (TGF β) induced fibroblast [13, 14].

Future research is mostly focusing on disease modification and prevention; hence, this work focuses on the protective therapy of nintedanib on diabetic nephropathy as it suppresses signaling pathways such as TGF-β/Smad2/3, P38MAPK (mitogen activated protein kinase), that are involved in the development of diabetic nephropathy. It may show a therapy for renal complications associated with diabetes [15].

2. MATERIALS AND METHODS

Healthy adult albino wistar rats of weighing 150-250 g were obtained from central animal house of Swamy Vivekananda College of Pharmacy, Elayampalayam, and Namakkal-637 205. Throughout the acclimatization and experimental period, the animals were housed in polypropylene cages (six rat per cage) in standard environmental lighting condition of (12-hr light/ 12-hr dark cycle), maintained under controlled room temperature (23 ± 2°C), and a relative humidity of 60% ± 10%. Rats were provided with standard pellet diet and water ad libitum freely throughout the study. The cages were cleaned daily by changing the husk bedding. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Swamy Vivekananda College of Pharmacy, Elayampalayam, Namakkal-637205. The animals were acclimatized for 2 weeks in the laboratory conditions before experiment. According to the Indian National Science Academy's criteria for the use and care of experimental animals in research, all of the study's animals were treated humanely, and all animal studies will be conducted in accordance with CCSEA regulations.

2.1. IAEC Reference No: SVCP/IAEC/PG/4/07/2024

2.2 Animal Grouping

S. No	Groups	Treatment
1.	Group-I (Normal control)	Normal saline
2.	Group-II (Disease control)	Streptozotocin (45mg/kg *i.p*) + Nicotinamide (100 mg/kg .*i.p*.)
3.	Group-III (Standard treatment)	Streptozotocin + Nicotinamide + Metformin (100 mg/kg,*p.o*.)
4.	Group-IV (Test treatment at low dose)	Streptozotocin + Nicotinamide + Nintedanib (30 mg/kg, *p.o*.)
5.	Group-V (Test treatment at high dose)	Streptozotocin + Nicotinamide + Nintedanib (50 mg/kg, *p.o*.)

2.3 Induction of Diabetic Nephropathy

Streptozotocin (STZ) (45 mg/kg) produced freshly in ice-chilled 0.1 M citrate buffer ($_P$H-4.5) was injected intraperitoneally once to induce diabetic nephropathy in overnight-fasted rats. After 15 minutes, nicotinamide (100 mg/kg) in 0.9% normal saline was given. Overnight, the animals were given a 2% glucose solution to counteract the hypoglycemic impact of the medication. Hyperglycemia in rats was confirmed through the elevated glucose levels (> 250 mg/dL) in the blood, determined at 72 hours of injection. Blood glucose level was measured using glucometer [16, 17].

The rats were divided into five groups comprising six animals in each group. Each group underwent a different treatment protocol. Group I serves as a normal control, received normal saline. Group II serves as a disease control, received streptozotocin (45 mg/kg) and nicotinamide (100 mg/kg) intraperitonealy. Group III serves as a standard group is treated with metformin (100 mg/kg, *p.o*.). Group IV were treated with nintedanib (30 mg/kg, *p.o*) in 0.9% normal saline. Group V were treated with nintedanib (50 mg/kg, *p.o*) in 0.9% normal saline. All animals had free access to regular rat chow and drinking water during experimental period.

2.4 Physical Evaluation

2.4.1 Measurement of Body Weight

Body weight of each rat in all groups was measured weekly once till the end of treatment using weighing balance and the changes were recorded [18].

2.4.2 Measurement of Feed Intake

Feed consumption of each rat in all groups was measured daily till the end of treatment using weighing balance and the changes were recorded [19].

2.4.3 Measurement of Water Intake

Water consumption of each rat in all groups was measured daily till the end of treatment using measuring cylinder and the changes were recorded.

2.5 Estimation of Blood Glucose Level

Blood glucose level (BGL) was measured using an Accu-Check blood glucometer on the 0th, 3rd, 7th, 14th, 21st, and 28th days and recorded, and an average value was taken. Blood from rats was withdrawn aseptically from the tail veins of each animal.

2.6 Hematological Estimation

On the end of study the blood was collected through retro orbital puncture for examination of

glycated haemoglobin (HbA1C) level. And the percentage of total glycated hemoglobin was recorded.

2.7 Biochemical Estimation

2.7.1 Serum Analysis

Blood samples from the animals were collected by retro orbital puncture from anaesthetized rat into clean heparinized bottles. The serum was separated from the blood and centrifuged according to groups into clean bottles for biochemical analysis. BUN (Blood urea nitrogen), creatinine, uric acid and albumin were analyzed according to the reported methods.

2.7.2 Urine Analysis

At the end of study, all rats were kept in individual metabolic cages and urine samples of 24 hours were collected. Rats were allowed to drink water during the urine collection period. The urinary parameters like creatinine, uric acid, total protein were analyzed according to the reported methods [20].

2.8 Measurement of Kidney Weight

At the end of the study, all rats were sacrificed with anaesthesia and kidneys were removed and compared with the control groups of rats. The weight was measured by using digital balance; the changes in the weight of kidneys were recorded.

2.10 Statistical Analysis

The data represented as Mean ± SEM of six replicated determinations. Results were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison. The difference was considered significant when $p<0.05$. All statistical tests were carried out using Prism 9.0 (Graph Pad, San Diego CA, USA) statistical software.

3. RESULTS

3.1 Effect of Nintedanib on Body Weight against Diabetic Nephropathy in Rats

Body weights of each rat in all groups were measured weekly till the end of the treatment using weighing balance and changes are recorded. In our study the body weight of the rats in disease control were decreased when compared to normal control. The body weight ofrats in the groups treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly increased when compared to the disease control group.

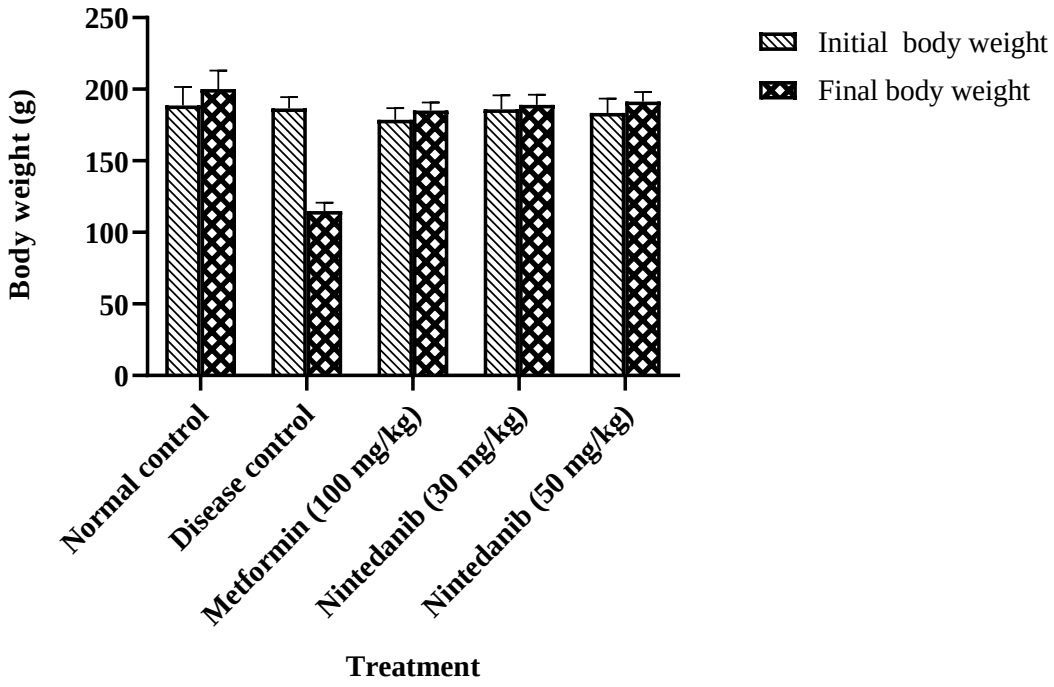

Figure 1: Graphical Representation of Effect of Nintedanib on Body Weight against Diabetic Nephropathy in Rats

3.2 Effect of Nintedanib on Feed Intake against Diabetic Nephropathy in Rats

Feed intake of rats in each groups were measured daily and the changes were recorded. In our study the feed intake of rats in disease control significantly increased when compared to the normal control and the feed intake of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control.

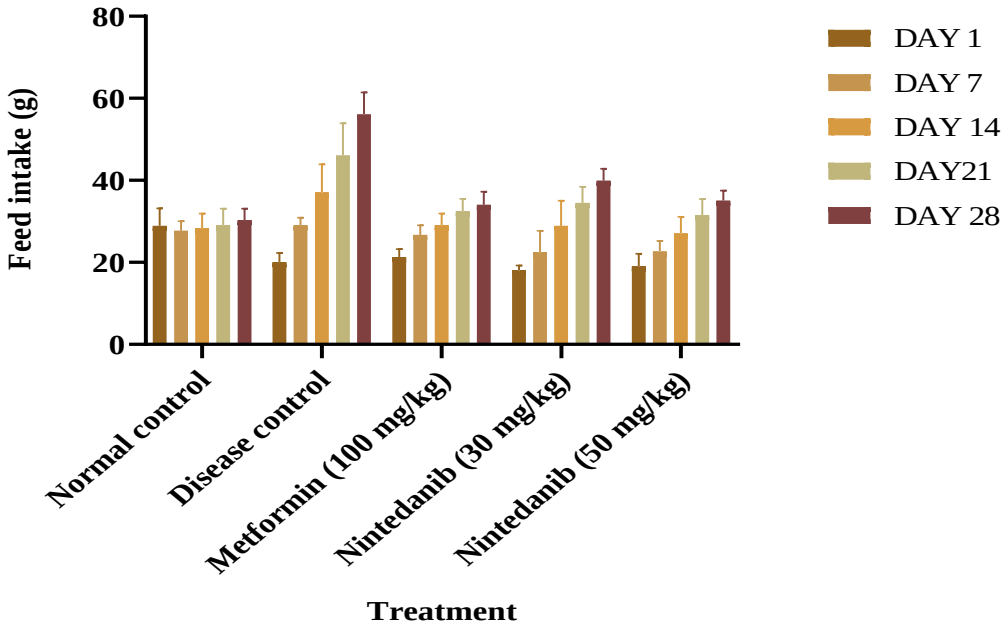

Figure 2: Graphical Representation of Effect of Nintedanib on Feed Intake against Diabetic Nephropathy in Rats

3.3 Effect of Nintedanib on Water Intake against Diabetic Nephropathy in Rats

Water intake of rats in each groups were measured daily and the changes were recorded. In our study the water intake of rats in disease control significantly increased when compared to the normal control and the water intake of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control.

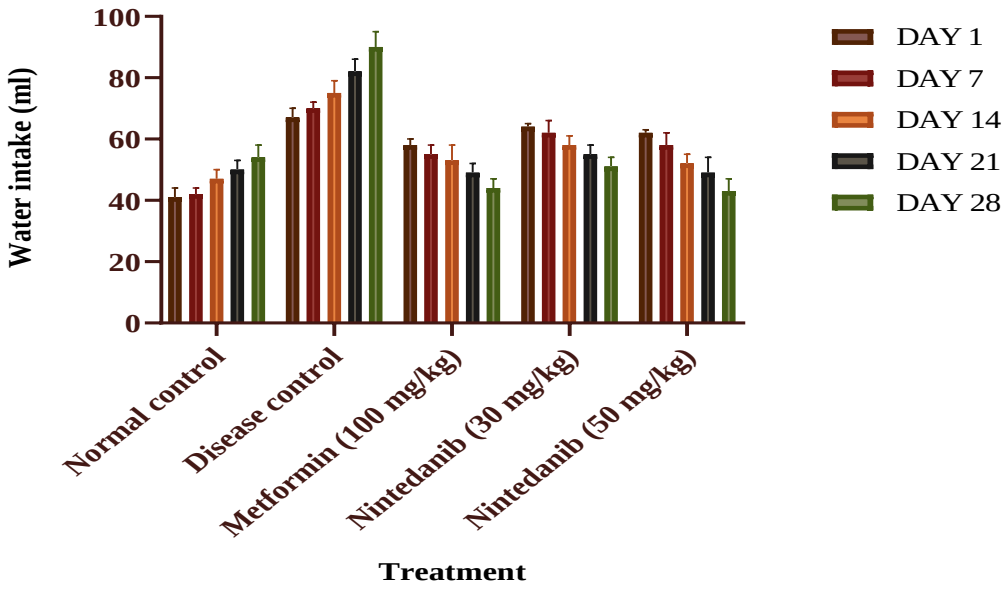

Figure 3: Graphical Representation of Effect of Nintedanib on Water Intake against Diabetic Nephropathy in Rats

3.4 Effect of Nintedanib an Blood Glucose Level Against Diabetic Nephropathy Rats

The blood glucose levels of rats in disease control group were significantly increased on day 28 when compared to the normal control and the blood glucose levels of rats that are treated with Metformin (100 mg/kg), were significantly decreased and treatment with Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were slightly decreased when compared to disease control group.

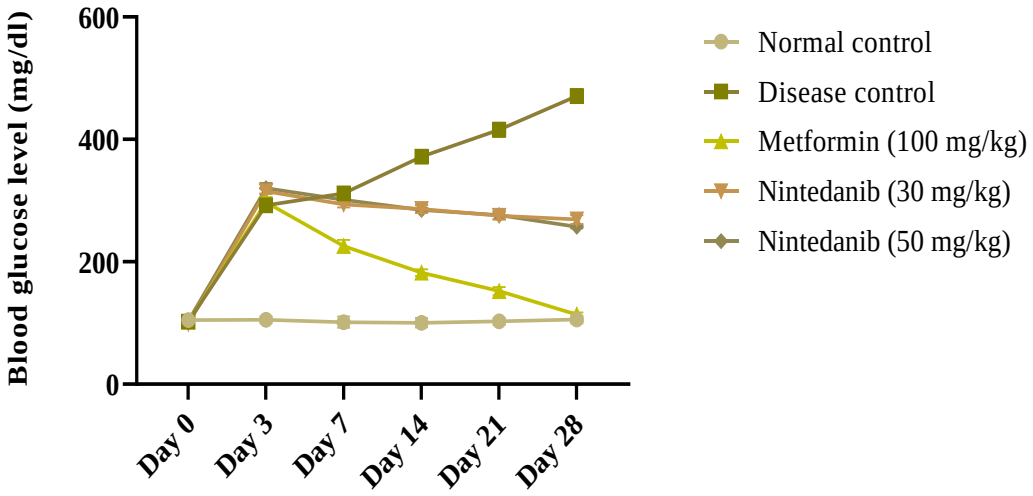

Figure 4: Graphical Representation of Effect of Nintedanib on Blood Glucose against Diabetic Nephropathy in Rats

3.5 Effect of Nintedanib on Glycated Hemoglobin (HbA1c) Level against Diabetic Nephropathy in Rats

The HbA1c level of rats in disease control group were significantly increased when compared to the normal control and the HbA1c levels of rats that are treated with Metformin (100 mg/kg) were significantly decreased and treatment with Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were slightly decreased when compared to disease control group.

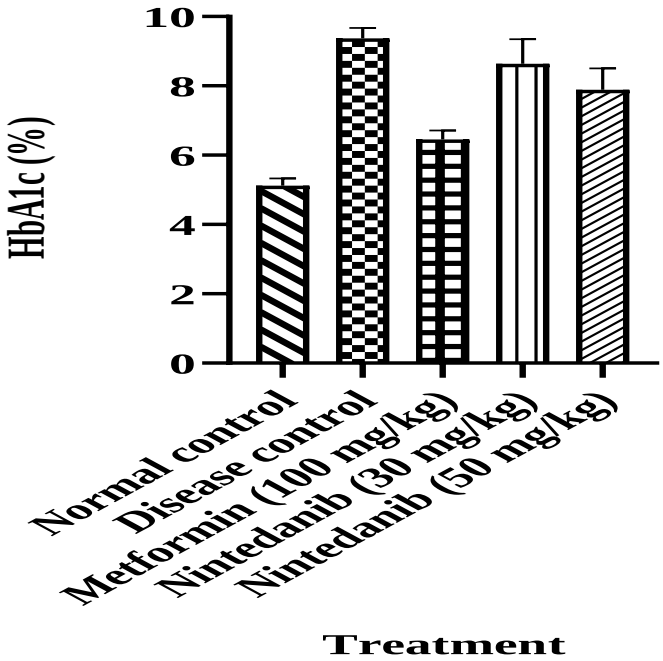

Figure 5: Graphical Representation of Effect of Nintedanib on Hba1c Level against Diabetic Nephropathy in Rats

3.6 Effect of Nintedanib on Serum Creatinine against Diabetic Nephropahty in Rats

The serum creatinine levels of rats in disease control group were significantly increased when compared to the normal control and the serum creatinine levels of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control.

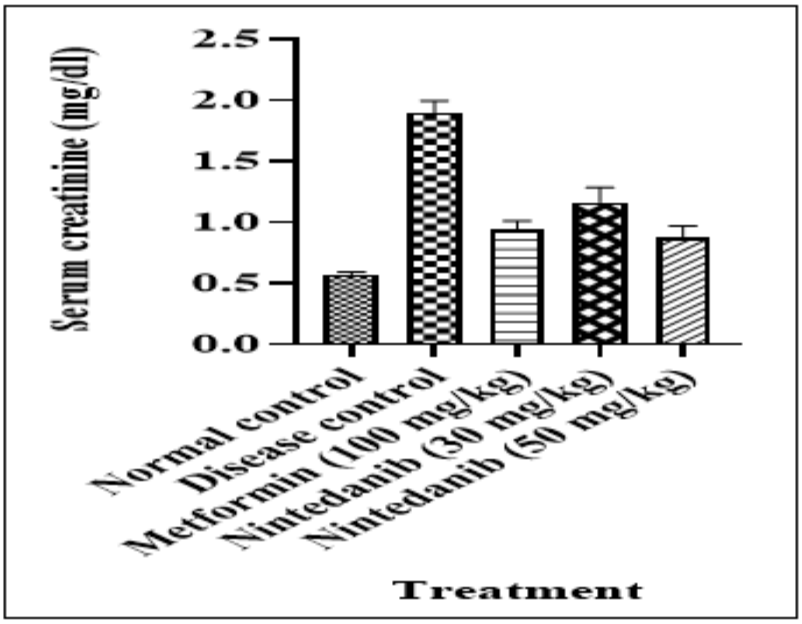

Figure 6: Graphical Representation of Effect of Nintedanib on Serum Creatinine against Diabetic Nephropathy in Rats

3.7 Effect of Nintedanib on Blood Urea Nitrogen (BUN) Against Diabetic Nephropahty in Rats

The serum BUN levels of rats in disease control group were significantly increased when compared to the normal control and the serum BUN levels of rats that are treated with Metformin (100 mg/kg) Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control group.

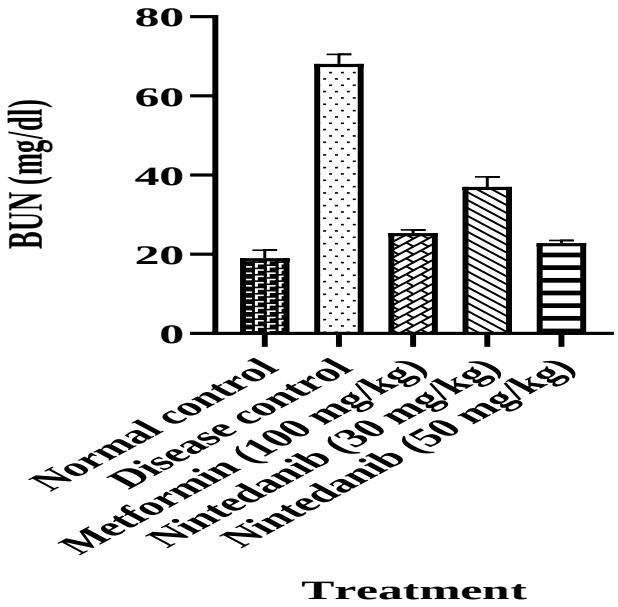

Figure 7: Graphical Representation of Effect of Nintedanib on BUN against Diabetic Nephropathy in Rats

3.8 Effect of Nintedanib on Serum Albumin against Diabetic Nephropahty in Rats

The serum albumin levels of rats in disease control group were significantly decreased when compared to the normal control and the serum albumin levels of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly increased when compared to disease control group.

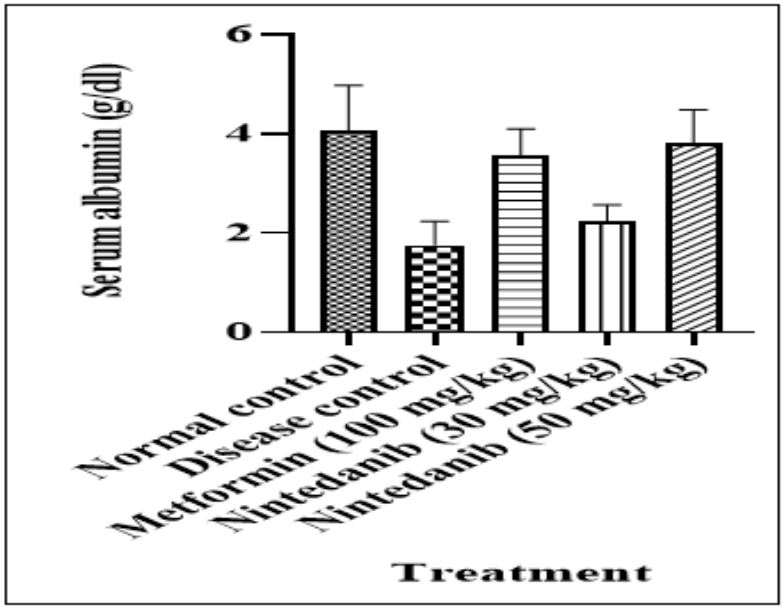

Figure 8: Graphical Representation of Effect of Nintedanib on Serum Albumin against Diabetic Nephropathy in Rats

3.9 Effect of Nintedanib on Urine Creatinine against Diabetic Nephropathy in Rats

The urine creatinine levels of rats in disease control group were significantly decreased when compared to the normal control and the urine creatinine levels of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly increased when compared to disease control group.

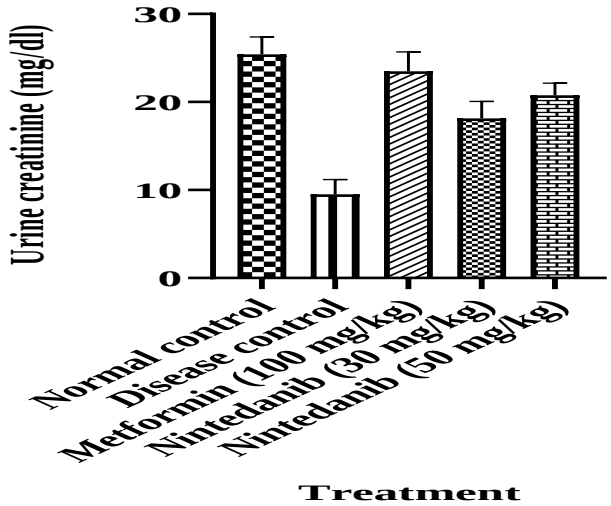

Figure 9: Graphical Representation of Effect of Nintedanib on Urine Creatinine against Diabetic Nephropathy in Rats

3.10 Effect of Nintedanib on Urine Total Protein and Microalbuminuria against Diabetic Nephropathy in Rats

1) Total protein

The urine total protein levels of rats in disease control group were significantly increased when compared to the normal control and the urine albumin levels of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control group.

2) Microalbuminuria

The microalbuminuria levels of rats in disease control group were significantly increased when compared to the normal control and the microalbuminuria levels of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control group.

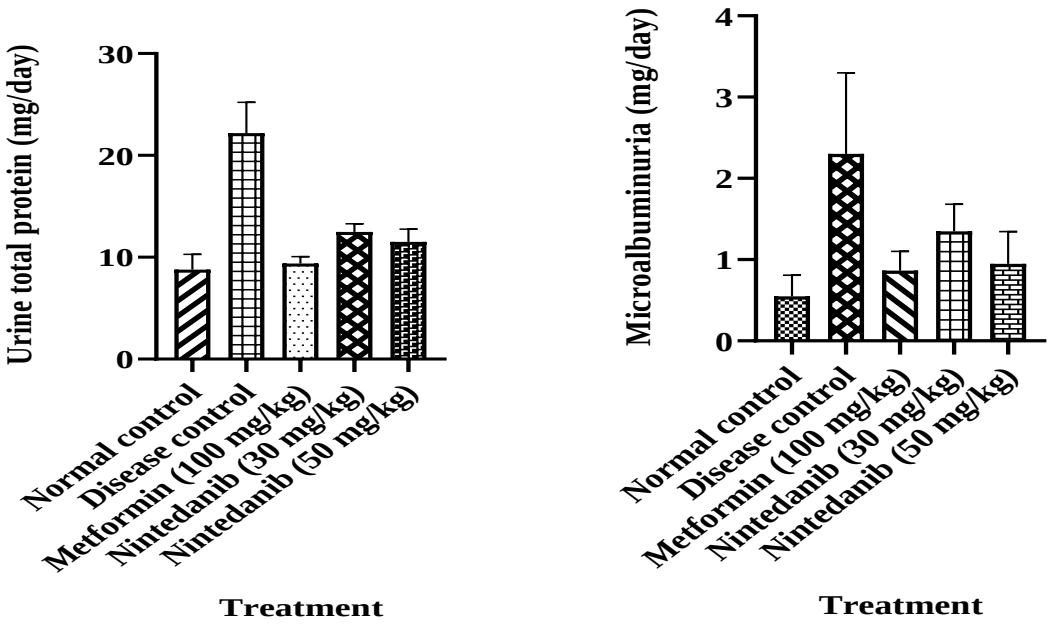

Figure 11: Graphical Representation of Effect of Nintedanib on Urine total protein and Microalbuminuria against Diabetic Nephropathy in Rats

3.11 Effect of Nintedanib on Uric Acid against Diabetic Nephropathy in Rats

The uric acid levels in urine of rats in disease control group were significantly increased when compared to the normal control and the urine uric acid levels of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control group.

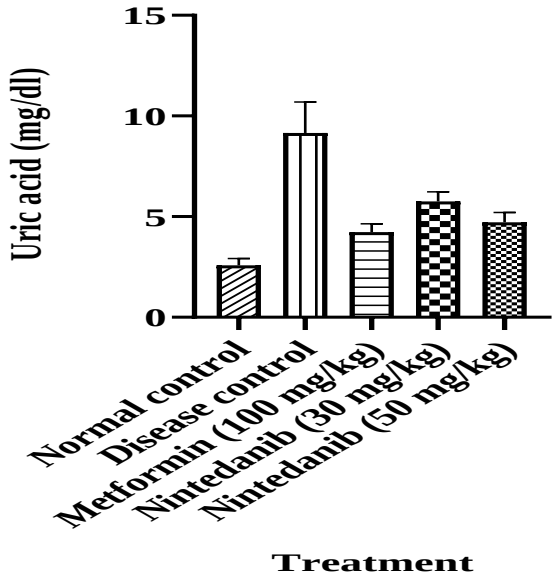

Figure 12: Graphical Representation of Effect of Nintedanib on Uric Acid against Diabetic Nephropathy in Rats

3.12 Effect of Nintedanib on Kidney Weight against Diabetic Nephropathy in Rats

The weight of kidney on the rats in disease control group were significantly increased when compared to the normal control and the kidney weights of rats that are treated with Metformin (100 mg/kg), Nintedanib (30 mg/kg) and Nintedanib (50 mg/kg) were significantly decreased when compared to disease control group.

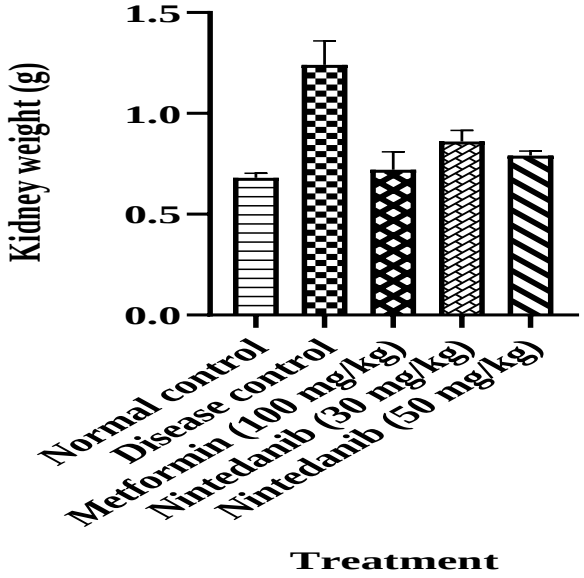

Figure 13: Graphical Representation of Effect of Nintedanib on Kidney Weight against Diabetic Nephropathy in Rats

4. DISCUSSION

Our findings has shown decrease in body weight and muscle mass as a result of losing protein through their urine in diabetic rats, as in compliance with previous studies. The administration of 30 and 50 mg/kg of nitedanib daily slowed the reduction of body weight in diabetic rats'. Furthermore, the feed and water intake in diabetic rats gradually increased due to polyphagia and polydipsia. Treatment with nintedanib (30 and 50 mg/kg per day) showed balanced feed and water intake as that of the normal control.

High glucose uptake by renal cells under hyperglycemic conditions can stimulate multiple pathological pathways, including the PKC pathway, which in turn causes increased expression of TGF-β and VEGF, which consequently causes mesangial expansion and glomerular enlargement and leads to the progression of diabetic nephropathy.

The results of the renal function parameters indicated that the disease control group had higher serum creatinine and BUN levels. This could be because of the extracellular matrix accumulation in tubules caused by TGF-β that damage kidney blood vessels, resulting in glomerulosclerosis and a decline in kidney function. This eventually decreases the kidneys' capacity to filter waste products like creatinine and reduces glomerular filtration rate and tubular secretion. It also causes the deposition of waste materials in the blood, notably nitrogenous compounds, resulting in higher serum creatinine and BUN levels and decreased creatinine levels in the urine. Treatment with nintedanib (30 and 50 mg/kg per day) resulted its ability to restore kidney function to normal by lowering serum creatinine and BUN levels and increasing urine creatinine levels. When compared to disease control group, the treatment with nintedanib (50 mg/kg) has shown effective results than that of the treatment with nitedanib (30 mg/kg).

The serum albumin level was lower in the disease control group when compared to normal control group due to increased glomerular permeability through the accumulation of extracellular matrix, which damages the blood vessels of the kidney and allows proteins to leak from the bloodstream into the urine, which also leads to increased protein excretion in the urine. Treatment with nintedanib (30 and 50 mg/kg per day) retains the decreased level of serum albumin and also decreases the excretion of protein and albumin in urine compared to disease control. In comparison between nintedanib (30 mg/kg) and nintedanib (50 mg/kg), treatment with nintedanib (50 mg/kg) has shown a better effect.

Reduction in the amount of uric acid clearance by the kidneys is a possible outcome of diabetic nephropathy as it develops renal function impairment. The disease control group has resulted in elevated blood and urine uric acid levels could be the consequence of alterations in renal tubular reabsorption. On treatment with nintedanib (50 mg/kg) the level of uric acid excretion in urine has been significantly decreased.

In this study, the weight of kidneys has increased in diabetic nephropathy-induced rats when compared with other groups of rats. This may be the result of structural modifications to the kidney due to hyperfiltration, which induces enlargement of the kidney, increases the workload on the kidneys, and causes hypertrophy and fluid retention that result in kidney swelling and edema in nephropathy. Increased extracellular matrix accumulation, including collagen

and fibronectin, also contributes in increased kidney weight. Since the treatment with nintedanib decreased weight of kidney significantly when compared to the disease control group, this suggests that the treatment prevents the structural changes of kidney due to nephropathy. On treatment with nintedanib (50 mg/kg) has a greater effect than nintedanib (30 mg/kg).

5. CONCLUSION

The present study's results offer strong support for nintedanib's renoprotective benefits on diabetic nephropathy. Compared to the disease control group, treatment with nintedanib leads to a substantial improvement in renal function parameters, such as decreased serum creatinine, BUN levels, and urine protein excretion, as well as tends to increase urine creatinine excretion and serum albumin levels. Thereby, nintedanib at 50 mg/kg has demonstrated better effectiveness than nintedanib at 30 mg/kg. These findings supports that nintedanib at exerts its renoprotective effects by inhibiting TGF-β, vascular endothelial growth factor, and possibly targeting multiple signaling pathways involved in the pathogenesis of diabetic nephropathy. Our finding suggests that nintedanib holds potential as a novel therapeutic agent for the management of diabetic nephropathy. While the results of our preclinical study are promising, to assess nintedanib's long-term safety and effectiveness in clinical therapies, as well as to clarify the precise processes underlying its impact on diabetic nephropathy, more research is required.

REFERENCES

1. Cole, J. B., & Florez, J. C. (2020). Genetics of diabetes mellitus and diabetes complications. *Nature reviews nephrology*, *16*(7), 377-390.
2. Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. I. D. F. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes research and clinical practice*, *138*, 271-281.
3. Sheela, N., Jose, M. A., Sathyamurthy, D., & Kumar, B. N. (2013). Effect of Silymarin on Streptozotocin-Nicotinamide--induced Type 2 Diabetic Nephropathy in Rats. *Iranian journal of kidney diseases*, *7*(2).
4. Verzola, D., Gandolfo, M. T., Ferrario, F., Rastaldi, M. P., Villaggio, B., Gianiorio, F., ... & Garibotto, G. (2007). Apoptosis in the kidneys of patients with type II diabetic nephropathy. *Kidney international*, *72*(10), 1262-1272.
5. Allen, D. A., Harwood, S. M., Varagunam, M., Raftery, M. J., & Yaqoob, M. M. (2003). High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases. *The FASEB journal*, *17*(8), 1-21.
6. Jones, C. A., Krolewski, A. S., Rogus, J., Xue, J. L., Collins, A., & Warram, J. H. (2005). Epidemic of end-stage renal disease in people with diabetes in the United States population: do we know the cause?. *Kidney international*, *67*(5), 1684-1691.
7. Brown, W. V. (2008). Microvascular complications of diabetes mellitus: renal protection accompanies cardiovascular protection. *The American journal of cardiology*, *102*(12), 10L-13L.
8. Yamagishi, S. I., & Imaizumi, T. (2005). Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. *Current pharmaceutical design*, *11*(18), 2279-2299.
9. Yamagishi, S. I., Fukami, K., Ueda, S., & Okuda, S. (2007). Molecular mechanisms of diabetic nephropathy and its therapeutic intervention. *Current drug targets*, *8*(8), 952-959.
10. Ackermann, M., Kim, Y. O., Wagner, W. L., Schuppan, D., Valenzuela, C. D., Mentzer, S. J., ... & Konerding, M. A. (2017). Effects of nintedanib on the microvascular architecture in a lung fibrosis model. *Angiogenesis*, *20*, 359-372.
11. Roth, G. J., Binder, R., Colbatzky, F., Dallinger, C., Schlenker-Herceg, R., Hilberg, F., ... & Kaiser, R. (2015). Nintedanib: from discovery to the clinic. *Journal of medicinal chemistry*, *58*(3), 1053-1063.
12. Verzola, D., Gandolfo, M. T., Ferrario, F., Rastaldi, M. P., Villaggio, B., Gianiorio, F., ... & Garibotto, G. (2007). Apoptosis in the kidneys of patients with type II diabetic nephropathy. *Kidney international*, *72*(10), 1262-1272.
13. Alicic, R. Z., Rooney, M. T., & Tuttle, K. R. (2017). Diabetic kidney disease: challenges, progress, and possibilities. *Clinical journal of the American Society of Nephrology*, *12*(12), 2032-2045.
14. Hill, N. R., Fatoba, S. T., Oke, J. L., Hirst, J. A., O'Callaghan, C. A., Lasserson, D. S., & Hobbs, F. R. (2016). Global prevalence of chronic kidney disease–a systematic review and meta-analysis. *PloS one*, *11*(7), e0158765.
15. Mestry, S. N., Dhodi, J. B., Kumbhar, S. B., & Juvekar, A. R. (2017). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. *Journal of traditional and complementary medicine*, *7*(3), 273-280.
16. Sathibabu Uddandrao, V. V., Brahmanaidu, P., Ravindarnaik, R., Suresh, P., Vadivukkarasi, S., & Saravanan, G. (2019). Restorative potentiality of S-allylcysteine against diabetic nephropathy through attenuation of oxidative stress and inflammation in streptozotocin–nicotinamide-induced diabetic rats. *European journal of nutrition*, *58*, 2425-2437.
17. Chinnappan, S. M., George, A., Thaggikuppe, P., Choudhary, Y., Choudhary, V. K., Ramani, Y., & Dewangan, R. (2019). Nephroprotective effect of herbal extract Eurycoma longifolia on paracetamol-induced nephrotoxicity in rats. *Evidence-Based Complementary and Alternative Medicine*, *2019*(1), 4916519.

18. Gocht, A., Spriewald, B., Distler, J. H., Ramsperger-Gleixner, M., Ensminger, S. M., Weyand, M., & Heim, C. (2018). Small molecule tyrosine kinase inhibitor nintedanib reduces development of cardiac allograft vasculopathy in murine aortic allografts. *Transplantation Direct*, *4*(7), e367.
19. Pachiappan, S., Murugaiyan, S., & Chandrasekar, S. (2021). Emerging intervention of antidepressant with DMARD in non-cancerous nociceptive persistent pain associated depression in FCA induced rheumatoid arthritic rats. *Brazilian Journal of Pharmaceutical Sciences*, *56*, e18852.
20. Sabarinath, C., Gayathridevi, S., Sudhakar, P., Sariga, V., Sneha, S., & Gayathiri, M. (2024). Evaluation of Anti-Urolithiasis Activity of Leucasaspera Byin Vitro Method. *Asian Journal of Pharmaceutical Research and Development*, *12*(5), 1-7.