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"EVALUATION OF NEPHRO PROTECTIVE EFFECT OF NICORANDIL IN STREPTOZOTOCIN-INDUCED NEPHROPATHY IN DIABETIC RATS"

Yogesh H S[1], Ranjitha C[2], Seema Gupta[3], K. S. Srilatha[4*], S C Marapur[5]

[1]NITTE College of Pharmaceutical Sciences, Bengaluru, Karnataka, India.
[2]Al-Ameen College of Pharmacy Bengaluru, Karnataka, India.
[3]Mallige College of Pharmacy Bengaluru, Karnataka, India.
[4]R.R.College of Pharmacy, Chikkabanavara, Bengaluru, Karnataka, India.*
[5]BLDEA'S SSM college of Pharmacy and Research Centre Vijayapura Karnataka, India

***Corresponding Author**: K.S.Srilatha*
**Assoc prof, Dept of Pharmaceutics, R.R.College pharmacy Bengaluru*

Abstract

BACKGROUND: One of the main microvascular complications of diabetes that leads to end-stage renal disease and the need for kidney dialysis or transplantation is diabetic nephropathy. Neither the kidney damage caused by diabetic nephropathy nor its prevention can be reversed with medication. The goal of the current study was to assess nicorandil's impact on diabetic rats' nephropathy caused by streptozotocin (STZ).
METHOD: Male Wistar rats that had fasted for the whole night were given 50 mg/kg intraperitoneally (i.p.) of streptozotocin to induce diabetes. Rats with fasting blood glucose levels more than 250 mg/dl were classified as diabetics and placed into four groups following a 48-hour streptozotocin treatment. The first two groups were maintained as controls for diabetes and normal conditions, respectively. After inducing diabetes, treatment groups received oral doses of nicorandil (2.5 and 5 mg/kg). These doses were given for six weeks. Serum glucose, glycosylated haemoglobin, serum albumin, serum creatinine, serum total protein, serum blood urea nitrogen, urine albumin, urine creatinine excretion rate, creatinine clearance, kidney index, antioxidant levels in kidney homogenate, MDA levels, and renal histopathology were measured at the conclusion of the six-week Nicorandil treatment.
RESULTS: When compared to the diabetic control group, the insulin-treated diabetic rats exhibited lower serum glucose and glycosylated haemoglobin levels. When compared to diabetic rats, the diabetic treatment rats demonstrated a significant decrease in blood urea nitrogen, serum creatinine, urinary albumin, haemoglobin, kidney index, and increased levels of catalase, superoxide dismutase, glutathione, nitric oxide, and malondialdehyde. Diabetic treatment rats also demonstrated a significant increase in body weight, serum albumin, creatinine

clearance, serum total proteins, and urinary creatinine. The kidney's histopathological results provided additional evidence for the preventive role of nitric oxide supplementation in reducing renal damage**.**
CONCLUSION: Nicorandil demonstrates good effect of renal protection in diabetic rats and so can be a promising drug for treatment and prevention of diabetic nephropathy.

KEYWORDS: Nicorandil, diabetic nephropathy, Streptozotocin.

2 INTRODUCTIONS

Hyperglycemia is a common metabolic disease that is shared by a range of disorders collectively referred to as diabetes mellitus. The chronic metabolic condition known as diabetes mellitus is typified by the incapacity to keep blood glucose levels within physiological bounds. [1]. It is widely acknowledged that there are four main types of diabetes: type 1 and type 2, gestational diabetes, and diabetes brought on by other particular circumstances [2].

One of the main causes of morbidity and death in diabetics is diabetic nephropathy (DN). The primary symptoms of diabetic kidney disease (DN) include increased excretion of urine albumin, urea, uric acid, and creatinine; glomerular lesions; and decreased GFR. It is the main cause of end-stage renal failure that results from chronic kidney disease. Before albuminuria, decreased GFR, and the growth of mesangial cells, structural alterations in the kidney can be detected 2–8 years after the onset of diabetes [3,4]. According to reports, nephropathy develops in 30–40% of diabetic people and is now the primary cause of end-stage renal failure globally [5,] 6 major contributing factor to the development of diabetic nephropathy is the build-up of advanced glycation end products (AGEs) and inadequate regulation of blood sugar levels. Moreover, tissue damage linked to diabetic nephropathy has been linked to advanced glycation end products [7].

Nicorandil is a nicotinamide ester that acts pharmacologically through two different mechanisms: first, it causes the opening of potassium (KATP)-sensitive channels, which dilates peripheral and coronary resistance arterioles; second, the nitrate moiety of the drug dilates systemic veins and epicardial coronary arteries. Nicorandil thus lowers preload and afterload while increasing coronary blood flow. [8,9]

Not every patient with type 1 diabetes experiences nephropathy. Epidemiological studies indicate that after a mean of 15 years with diabetes, 40 percent of patients with type 1 diabetes develop diabetic nephropathy. Thus, a molecule that can halt the onset and advancement of nephropathy in diabetics is required. [10 11]. Strong anti-oxidant medication Nicorandil has been shown to provide excellent protection against oxidative stress in a number of circumstances, including the avoidance of oxidative stress in diabetes caused by streptozotocin.[12].

3 **Materials**

Experimental Animals

The study employed adult male Wistar rats weighing between 250 and 300 g. The Institutional Animal Ethics Committee, or IAEC, approved the use of animals in these experiments (AACP/IAEC/March/2019/03). Rats used in the experiment were handled in compliance with CPCSEA guidelines at all times.

3.1 Method

3.1 a Streptozotocin (50 mg/kg) dissolved in freshly prepared ice cold sodium citrate buffer(0.802 g of anhydrous citric acid and 1.712 g of sodium citrate di-hydrate is dissolved in 100ml of water) for inducing diabetes.

3**.1. b** The drug Nicorandil was used by dissolving in water for the treatment. The solutions were freshly prepared every day before dosing the animals.

3.2 Experimental design and drug treatment

3.2.1 Induction of diabetes.

After an overnight fast, the rats were given 50 mg/kg body weight of streptozotocin intraperitoneally, which was dissolved in freshly made ice 0.1M sodium citrate buffer (pH 4.5). To prevent hypoglycemia, the rats were given a 10% w/v glucose solution 24 hours following the injection. After 48 hours, fasting blood glucose levels

were assessed. Rats that tested more than 250 mg/dL for fasting blood glucose were classified as diabetic and used in the investigation. Nicorandil (2.5 mg/kg, po) and Nicorandil (5 mg/kg, po) are the protective medications that are employed. Drug dosages for humans were converted to doses for animals by computing based on the weight of the animal [13].

3.2.2 Study protocol

To induce diabetes in the rats, a single intraperitoneal injection of streptozotocin 50 mg/kg was administered. For six weeks, the rats were administered the preventive medications Nicorandil (2.5 mg/kg) and Nicorandil (5 mg/kg) orally every day. Weight was measured once a week. Anaesthesia overdose resulted in the sacrifice of rats. Under light ether anaesthesia, blood was collected from the retro orbital plexus to test biochemical parameters. The kidney was also extracted for histological investigations and to assess the antioxidant activity of tissue homogenates.

3.3 Parameters assessed

3.3.1 Body weight:

Body weight of the rats was taken on a weighing balance once every week. Loss of body weight was compared with the body weight which was measured at the beginning and at the end of the study.

Figure1: Measurement of body weight using a digital weighing balance

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3.3.1.1 Collection of blood, dissection and homogenate.

After 6 weeks of treatment the rats were anaesthetized then blood was withdrawn from retro- orbital plexus. Blood was collected in EDTA storage tubes. Subsequent animals were sacrificed by over dose of anaesthesia and kidney was isolated.

3.3.1.2 Kidney index.

Kidneys were patted dry, put on butter paper, and weighed on an analytical balance to determine the wet weight prior to the manufacture of kidney homogenate. The renal weight was given as mg/g.

Fasting blood glucose level

Method: Initial rate assay

Assay principle:

Glucose is oxidised by glucose oxidase (GOD) to produce hydrogen peroxide and gluconic acid. Released hydrogen peroxide reacts with phenol and 4-aminopyrine (4-AAP) in the presence of the peroxidase enzyme to generate coloured quinone imine dye. The amount of glucose present in this sample was precisely proportionate to the absorbance of coloured dye, which was measured at 505 nm.

Estimation of glycosylated haemoglobin [15]

Assay principle:

Operationally speaking, glycosylated haemoglobin (GHb) is defined as the fast fraction haemoglobins HbA1 (Hb A1a, A1b, and A1c) that elute first in column chromatography. HbAo is the designation for the non-glycosylated form of haemoglobin, which makes up most of the haemoglobin.

A weekly binding cation-exchange resin is constantly mixed with a haemolyzed preparation of whole blood for five minutes. During both the hemolysate preparation and the binding, the labile fraction is removed. While this is being mixed. GHb is left free in the supernatant when HbAo binds to the ion exchange resin. The resin

is extracted from the supernatant using a filter separator following the mixing time. By comparing the absorbances of the total haemoglobin (THb) fraction and the glycosylated haemoglobin (GHb) fraction, the percentage of glycosylated haemoglobin can be ascertained. The percentage of glycosylated haemoglobin in the sample is determined by dividing the absorbances of the total haemoglobin fraction in the test and the control by the ratio of these two parameters.

3.3.1.3 Estimation of serum albumin [16]

Method: Bromo Cresol Green (BCG) dye, End point assay

Assay Principle:
At pH 3.68, Albumin acts as cation and binds selectively to the anionic dye bromocresol green, forming a green coloured complex. The absorbance of final colour was measured at 630 nm. The increase in absorbance of the resulting Albumin-dye complex was proportional to the Albumin concentration in the sample.

3.3.1.4 Estimation of Serum Total Proteins

3.3.1.5 Method: Modified Biuret, End point assay

3.3.1.6 Assay principle:
Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration.

$$\text{Protein} + \text{Cu}^{++} \xrightarrow{\text{Alkaline medium}} \text{Cu-protein complex}$$

3.3.1.6 Estimation of serum creatinine

Method: Jaffe's reaction, initial rate assay

Assay Principle:
In an alkaline media, creatinine and picric acid combine to generate an orange-colored complex. By monitoring the change in absorbance at 505 nm over a predetermined period of time, the rate of complex formation is determined, and it is directly correlated with the creatinine concentration. To prevent interference from ketoacids, the reaction time and sodium hydroxide and picric acid concentrations have been optimised.

$$\text{Creatinine} + \text{Picric acid} \xrightarrow{\text{Alkali}} \text{Creatinine–Picrate Complex (orange colour)}$$

3.3.1.7 Estimation of serum blood urea nitrogen [17]

Method: Berthelot, End point

Assay Principle:
Ammonia and carbon dioxide are produced when urea is hydrolyzed in the presence of water and urease. The resulting ammonia, which was detected at 578 nm (570–600 nm), combines with hypochlorite and phenolic chromogen under alkaline circumstances to generate green indophenols. As a catalyst, sodium nitroprusside works. The quantity of urea in the sample directly correlates with the color's intensity.

3.3.1.8 **Urine parameters**.
Collection of urine
Individual rats of each group were placed in metabolic cages with free access for food and water to obtain 24-h urine collections; later urine volume was measured and taken for albumin and creatinine estimation.

3.3.1.9 Estimation of urinary albumin
The methodology, materials, and assay parameters are the same as for serum albumin estimation; the only difference is that the urine sample was diluted to a ratio of 1:20 before estimate was performed.

3.3.1.10Estimation of urinary creatinine

With the exception of diluting the collected urine to a ratio of 1:10 before estimating, the methodology, materials, and test parameters were the same as those used in serum creatinine measurement.

3.3.1.11 Creatinine clearance and urinary albumin excretion rate [17]

An indicator of glomerular filtration rate (GFR) called creatinine clearance (CrCl) was determined using the equation CrCl= UV/P×1440, where U is the urine creatinine (μ/L). P is serum creatinine (μ mol/L), and V is urine volume per day (ml/24 hours). The millilitres per minute (ml/minute) of creatinine clearance.

3.3.1.12 Urinary albumin excretion rate [18]

The formula for calculating urinary albumin excretion rate (UAR) (mg/24h) is as follows: 24 hours urine volume × urinary albumin (mg/dl). This indicates albuminuria.

3.3.1.13 Tissue estimation

Method of collection of kidneys

Rats were put to sleep using a large dosage of ether anesthesia, and the kidneys were removed fast after. A freezing-cold saline physiological solution that was kept at -200C was used to wash the kidneys. The kidneys were cleaned, weighed, coarsely chopped, and homogenized in order to estimate the amount of antioxidant enzymes. For histopathological investigations, the left kidney was preserved in 10%v/v neutralized buffered formalin.

Preparation of kidney homogenate

On ice, tissue was cut into tiny pieces. After preparing a 10% w/v homogenate in 10 mM phosphate buffer (pH7.4), the mixture was centrifuged at 13,000×g for 10 minutes at 40°C. The assessment of antioxidant enzyme activity was conducted using the supernatant.

3.3.1.14 Estimation of catalase activity in kidney homogenate [17]

It is a quantitative spectroscopic technique designed to track the H_2O_2 breakdown at 240 nm over time in units of measurement for regular catalase kinetics research.

Estimation of superoxide dismutase activity in kidney homogenate [18].

1. To prepare the tissue homogenate, take 2% weight/volume of tissue, add 10% weight/volume of buffer, and blend.
2. A mixture of 0.1 ml of the sample, 0.5 ml of carbonate buffer (pH 9.7), 0.1 ml of EDTA (1x 10-1 M), and 1 ml of epinephrine (3x10-3 M) was prepared.
3. Over the course of three minutes, at 30-second intervals, the optical density of the generated adeno chrome was measured at 480 nm.
4. The findings were presented as a unit per milligramme of protein. The enzyme concentration needed to impede the chromogen generation by 50% for one minute was defined as one unit of enzyme activity.

3.3.1.15 Estimation of reduced glutathione levels in kidney homogenate[18].

1. Sulphosalicylic acid (4%), 1.0 ml, was briefly precipitated with 1.0 ml of sciatic nerve homogenate (10%).
2. The samples were centrifuged at 1200 g for 15 minutes at 4 oC after being stored at 4 oC for at least an hour.
3. The test mixture comprised 3.0 millilitres total with 0.1 millilitres of supernatant, 2.7 millilitres of phosphate buffer (0.1M, pH 7.4), and 0.2 millilitres of 5,5, dithiobis (2-nitro benzoic acid) (Ellman's reagent, 0.1mM, pH 8.0).At 412 nm, the yellow colour formed instantly, and the decreased GSH levels were expressed as μg/mg protein.

3.3.1.16 Measurement of lipid peroxidation in kidney homogenate [18] .

a. Tissue extracts were mixed separately with 1 ml TCA (20%), 2 ml TBA (0.67%) and heated for 1 h at 100ºC.

b. After cooling, the precipitate was removed by centrifugation. The absorbance of each sample was measured at 535 nm using a blank containing all the reagents except the sample.

c. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction coefficient of MDA, which is$1.56x\ 10^5\ M^{-1}\ cm^{-1}$ and were expressed as μ mol of MDA per mg protein.

3.4 Statistical analysis

The mean ± standard error of the mean was used to express all the data, which were then analysed using one way analysis of variance between the groups and Bonferroni's multiple comparison test.

3.5 Histopathology:

The left kidney is sent for histopathology examinations after being fixed in 10% v/v neutralised buffered formalin. These tissues were embedded in paraffin blocks, and thin slices measuring three to five micrometres were cut. These sections were then stained with hematoxylin-eosin and examined at a magnification of 100 times.

RESULTS

4.4.1 Effect of treatment with Nicorandil for six weeks on % change in body weight in STZ induced diabetic rats.

At the sixth week, the normal and diabetic rats' body weight changes were found to be 21.10 ± 1.969 and -50.64 ± 2.011, respectively. The body weight reduction percentage of diabetic rats was found to be significantly higher than that of normal rats. Similarly, the body weight change percentage of diabetic rats treated orally with 2.5 mg/kg and 5 mg/kg of nicorandil was found to be -41.55 ± 1.233 and -42.26 ± 1.438, respectively, which were significantly lower than that of diabetic rats.

Table 1.Effect of treatment with Nicorandil for six weeks on % change in body weight in diabetic rats.

Sl.no	Groups	Initial body weight(g)	Final body weight(g)	% change in body weight
01	Normal control	246.0 ± 3.838	297.7 ± 3.853	21.10 ± 1.969
02	Diabetic control	261.5 ± 5.220	128.8 ± 4.764	-50.64 ± 2.011$^{###}$
03	Diabetic +Nicorandil (2.5 mg/kg)	266.7 ± 4.410	155.7 ± 2.290	-31.55 ± 1.233***
04	Diabetic +Nicorandil (5 mg/kg)	258.0 ± 3.215	148.8 ± 3.114	-22.26 ± 1.438***

All values are expressed as mean ± SEM, n=6 analyzed by One-way Analysis of Variance (ANOVA) followed by *Bonferroni's Multiple Comparison Test,* $^{#}$P<0.05, $^{##}$P<0.01, $^{###}$P<0.001 Vs normal control group,*P<0.05, **P<0.01, ***P<0.001 Vs diabetic control group.

4.4.2 Effect of treatment with Nicorandil for six weeks on kidney index in STZ induced diabetic rats.

After six weeks, the kidney index of both normal and diabetic rats was measured. The kidney index of the diabetic rats was found to be 3.933 ± 0.01994, 9.593 ± 0.1248, respectively, and it was significantly higher than that of the normal rats. Similarly, the kidney index of the diabetic rats that received oral nicorandil treatment (2.5 mg/kg and 5 mg/kg) was found to be 7.277 ± 0.066, 6.257 ± 0.0 478, respectively, which was significantly lower than that of the diabetic rats.

Table 2: Effect of treatment with Nicorandil for six weeks on kidney index in diabetic rat

Sl. no	Groups	Kidney index (mg/g)
01	Normal control	3.933 ± 0.01994
02	Diabetic control	9.593 ± 0.1248$^{###}$
03	Diabetic + Nicorandil 2.5mg/kg	7.277 ± 0.066***
04	Diabetic + Nicorandil 5mg/kg	6.257 0.0478***

4.4.3 Effect of treatment with nicorandil for six weeks on % glycosylated haemoglobin in STZ induced rats.

Rats without diabetes and those with diabetes had GHb percentages of 3.139 ± 0.0134 and 19.52 ± 0.0650, respectively. When compared to normal rats, the percentage of GHb in diabetic rats was substantially higher. In comparison to diabetic rats, the percentage GHb of STZ-induced diabetic rats that received oral nicorandil

treatment (2.5 mg/kg) and nicorandil (5 mg/kg) was found to be significantly lower at 14.32 ± 0.040 and 10.16 ± 0.034, respectively.

Sl.no	Groups	%Glycosylated hemoglobin
01	Normal control	3.139 ± 0.013
02	Diabetic control	19.52 ± 0.065$^{###}$
03	Diabetic + Nicorandil 2.5mg/kg	14.32 ± 0.040***
04	Diabetic + Nicorandil 5mg/kg	10.16 ± 0.034***

Table 3: Effect of treatment with nicorandil for six weeks on % glycosylated hemoglobin in diabetic rats.

4.4.4 Effect of treatment with Nicorandil for sixth weeks on % change in serum glucose levels in STZ induced diabetic rats.

Rats with diabetes and normal blood glucose levels showed a % change in serum glucose levels of 4.613 ± 0.005 and 37.21 ± 0.048, respectively, which was significantly greater than the normal rats' serum glucose levels. When nicorandil (2.5 mg/kg) and nicorandil (5 mg/kg) were given orally to STZ-induced diabetic rats, the percentage change in serum glucose levels was determined to be -23.30 ± 0.005 and -40.25 ± 0.069, respectively. These results demonstrated a significantly reduced effect when compared to diabetic rats.

Table 4 : Effect of treatment for with phloroglucinol and micro nutritional supplement six weeks on % change in serum glucose in diabetic rats.

Sl no	Groups	At 0th day	At 6th day	% Change in serum glucose level
01	Normal control	97.13 ± 0.021	101.6 ± 0.088	4.613 ± 0.005
02	Diabetic control	326.0 ± 0.260	447.5 ± 0.076	37.21 ± 0.048$^{###}$
03	Diabetic + nicorandil 2.5mg/kg	334.5 ± 0.114	289.3 ± 0.088	-23.30 ± 0.056***
04	Diabetic + nicorandil 5 mg/kg	365.6 ± 0.012	266.3 ± 0.101	-40,25 ± 0.069***

4.4.5 Effect of treatment with nicorandil for six weeks on serum albumin levels in STZ induced diabetic rats.

Rats given STZ to induce diabetes had serum albumin levels of 1.988 ± 0.035 g/dl, which was considerably less than normal rats' values of 3.914 ± 0.1173 g/dl. After oral nicorandil treatment (2.5 mg/kg and 5 mg/kg), serum albumin levels in STZ-induced diabetic rats were reported to be 2.912 ± 0.0204 and 3.247 ± 0.043, respectively. These values were considerably higher than those of diabetic rats.

Table 5. Effect of treatment with nicorandil for six weeks on serum albumin levels in diabetic rats.

Sl.no	Groups	Serum albumin levels (gm/dl)
01	Normal control	3.914 ± 0.117
02	Diabetic control	1.988 ± 0.035$^{###}$
03	Diabetic + Nicorandil 2.5 mg /kg	2.912 ± 0.020***
04	Diabetic + Nicorandil 5 mg/kg	3.247 0.043***

4.4.6 Effect of treatment with Nicorandil for six weeks on serum creatinine levels in STZ induced diabetic rats.

Rats given STZ to induce diabetes had serum creatinine levels of 2.828±0.0172 mg/dl, which was considerably higher than the 0.7642±0.027 mg/dl values of normal rats. When compared to diabetic rats, the serum creatinine levels in STZ-induced diabetic rats that were given oral nicorandil at doses of 2.5 mg/kg and 5 mg/kg were reported to be 2.129 ± 0.024 g/dl and 1.462 ± 0.032 g/dl, respectively.

Table 6 : Effect of treatment with Nicorandil for six weeks on serum creatinine levels in diabetic rats.

Sl no.	Groups	Serum creatinine levels (mg/dl)
01	Normal control	0.764 ± 0.027
02	Diabetic control	2.828 ± 0.017$^{###}$

03	Diabetic + Nicorandil (2.5 mg/kg)	2.129 ± 0.024***
04	Diabetic + nicorandil (5 mg/kg)	1.462 0.032***

4.4.7 Effect of treatment with Nicorandil for sixth weeks on serum total proteins in STZ induced diabetic rats.

The total protein levels in the serum of STZ diabetic rats were found to be 3.897 ± 0.024 g/dl, a significantly lower amount than those of normal rats (8.697 ± 0.017 g/dl).The serum total protein levels of rats treated with nicorandil orally at 2.5 mg/kg and 5 mg/kg for STZ-induced diabetes were reported to be 4.932 ± 0.018 g/dl and 5.587 ± 0.021 g/dl, respectively. These values were considerably higher than those of diabetic rats.

Table 7. Effect of treatment with nicorandil for six weeks on serum total proteins in diabetic rats.

Sl.no	**Groups**	**Serum total proteins (gm/dl)**
01	Normal control	8.697 ± 0.017
02	Diabetic control	3.897 ± 0.024###
03	Diabetic +Nicorandil (2.5mg/kg)	4.932 ± 0.018***
04	Diabetic + Nicorandil (5mg/kg)	5.587 0.0216***

4.4.8 Effect of treatment with Nicorandil for six weeks on serum blood urea nitrogen levels in STZ induced diabetic rats.

Serum BUN levels in STZ induced diabetic rats was found to be 92.47 ± 0.139 mg/dl which was significantly higher than the serum BUN levels in normal rats (19.82 ± 0.048 mg/dl). Serum BUN levels in STZ induced diabetic rats orally treated with Nicorandil (2.5 mg/kg) and Nicorandil (5mg/kg) were found to be 75.50 ± 0.113mg/dl and 60.81 ± 0.295 mg/dl respectively, which was significantly reduced than that of diabetic rats.

Table 8 : Effect of treatment with nicorandil for six weeks on serum blood urea nitrogen levels in diabetic rats.

Sl.no	**Groups**	**Serum Blood urea nitrogen (mg/dl)**
01	Normal control	19.82 ± 0.048
02	Diabetic control	92.47 ± 0.139###
03	Diabetic +Nicorandil (2.5mg/kg)	75.50 ± 0.1135***
04	Diabetic + Nicorandil (5mg/kg)	60.81 0.295***

4.4.9 Urine estimation

Effect of treatment with Nicorandil for six weeks on urinary albumin levels in STZ induced diabetic rats.

The STZ diabetic rats had urine albumin levels of 16.44 ± 0.0143 g/dl, which was considerably higher than the normal rats' urinary albumin values of 2.647 ± 0.0105 g/dl.After oral nicorandil treatment (2.5 mg/kg and 5 mg/kg), the urinary albumin levels of STZ-induced diabetic rats were found to be considerably lower than those of diabetic rats, at 12.60 ± 0.0153 mg/dl and 9.643 ± 0.007 mg/dl, respectively.

Table 9. Effect of treatment with nicorandil for six weeks on urinary albumin levels in diabetic rats.

Sl.no	**Groups**	**Urinary albumin levels (g/dl)**
01	Normal control	2.647 ± 0.010
02	Diabetic control	16.44 ± 0.0143###
03	Diabetic + Nicorandil (2.5mg/kg)	12.60 ± 0.0153***
04	Diabetic + Nicorandil (5mg/kg)	9.643 0.0071***

4.4.10 Effect of treatment with nicorandil for six weeks on urinary creatinine levels in STZ induced diabetic rats.

Rats with STZ diabetes had urine creatinine levels of 3.420±0.2058 mg/dl, which was considerably less than normal rats' values (11.22±1.349 mg/dl).When compared to diabetic rats, the urinary creatinine levels of STZ-induced diabetic rats administered oral nicorandil at doses of 2.5 mg/kg and 5 mg/kg were reported to be 5.530 ± 0.1130 mg/dl and 7.687 ± 0.109 mg/dl, respectively.

Table 10. Effect of treatment with nicorandil for six weeks on urinary creatinine levels in diabetic rats.

Sl.no	Groups	Urinary creatinine level(mg/dl)
01	Normal control	9.193 ± 0.043
02	Diabetic control	2.505 ± 0.049$^{###}$
03	Diabetic + Nicorandil (2.5 mg/kg)	5.530 ± 0.113***
04	Diabetic + Nicorandil (5mg/kg)	7.697 0.109***

4.4.11 Effect of treatment with nicorandil for six weeks on creatinine clearance levels in STZ induced diabetic rats.

Creatinine clearance levels in STZ diabetic rats was 0.0636± 0.001 ml/min which was significantly lower than the urinary creatinine levels in normal rats (0.4124±0.0036ml/min). Creatinine clearance levels of STZ induced diabetic rats orally treated with nicorandil (2.5 mg/kg) and nicorandil (5mg/kg) were found to be 0.2752 ± 0.0021 ml/min and 0.3548 ± 0.0033 ml/min respectively, which were significantly increased when compared to that of diabetic rats.

Table 11: Effect of treatment with nicorandil for six weeks on creatinine clearance levels in STZ induced diabetic rats.

Sl.no	Groups	Creatinine Clearance Rate
01	Normal control	0.4124 ± 0.0036
02	Diabetic control	0.0636 ± 0.0010$^{###}$
03	Diabetic + Nicorandil (2.5 mg/kg)	0.2752 ± 0.0021***
04	Diabetic + Nicorandil (5mg/kg)	0.3548 0.0033***

4.4.12 Effect of treatment with nicorandil for six weeks on urinary albumin excretion rate (UAER) levels in STZ induced diabetic rats.

The STZ-induced diabetic rats had a urine albumin excretion rate of 3.408±0.004 µg/24h, which was considerably higher than the normal control group's urinary albumin excretion rate of 0.2235±0.0081 µg/24h.The oral treatment of rats with STZ-induced diabetes with 2.5 mg/kg and 5 mg/kg of nicotine resulted in significantly lower urinary albumin excretion rates (UAER): 2.517 ± 0.0035 µg/24 h and 2.163 ± 0.0080 µg/24 h, respectively.

Table 12 : Effect of treatment with nicorandil for six weeks on urinary albumin excretion rate (UAER) levels in STZ induced diabetic rats

Sl.no	Groups	Urinary albumin excretion rate
01	Normal control	0.2235 ± 0.008
02	Diabetic control	3.408 ± 0.004$^{###}$
03	Diabetic + Nicorandil (2.5 mg/kg)	2.517 ± 0.003***
04	Diabetic + Nicorandil (5mg/kg)	2.163 0.008***

4.4.13 Anti-oxidants levels:

Effect of treatment with Nicorandil for sixth weeks on SOD activity in kidney homogenate in STZ induced diabetic rats.

The SOD activity measured in the kidney homogenate of diabetic rats induced with streptozocin (STZ) was 71.93±1.662 U/min/g, a substantially lower value than that of the normal control group (209.1 ± 0.093 U/min/g). When compared to diabetic rats, the SOD activity of STZ-induced diabetic rats treated with nicorandil (2.5 mg/kg) and nicorandil (5 mg/kg) was found to be 138.3 ± 0.099 U/min /g and 154.4 ± 0.1563 U/min /g, respectively.

Table 13: Effect of treatment with Nicorandil for sixth weeks on SOD activity in kidney homogenate in STZ induced diabetic rats.

Sl.no	Groups	SOD Activity (units/min/g) in kidney homogenate
01	Normal control	209.1± 0.093
02	Diabetic control	71.93 ± 1.662$^{###}$
03	Diabetic +Nicorandil (2.5mg/kg)	138.3 ± 0.099***
04	Diabetic +Nicorandil (5mg/kg)	154.4 ± 0.156***

4.4.14 Effect of treatment with nicorandil for six weeks on catalase activity in kidney homogenate in STZ induced diabetic rats.

The study revealed that the kidney homogenate from diabetic rats induced with streptozocin (STZ) had considerably reduced levels of catalase activity (39.66±0.141 µmol/min/g) compared to the normal control group (71.33± 0.178 µmol/min/mg). When compared to diabetic rats, the catalase levels of STZ-induced diabetic rats receiving oral nicorandil (2.5 mg/kg) and nicorandil (5 mg/kg) were found to be significantly higher, at 49.56 ± 0.1305 µmol/min/g and 55.50 ± 0.142 µmol/min/g, respectively.

Table 14: Effect of treatment with nicorandil for six weeks on catalase activity in kidney homogenate in STZ induced diabetic rats.

Sl.no	Groups	Catalase activity in kidney homogenate
01	Normal control	71.33 ± 0.1782
02	Diabetic control	39. 66 ± 0.1412$^{###}$
03	Diabetic + Nicorandil (2.5 mg/kg)	49.56 ± 0.1305***
04	Diabetic + Nicorandil (5 mg/kg)	55.50 0.1429***

4.4.15 Effect of treatment with nicorandil for six weeks on GSH levels in kidney homogenate in STZ induced diabetic rats.

The renal homogenate of diabetic rats induced with streptozotocin (STZ) showed considerably lower levels of GSH (43.83±0.2152 nmol/mg) than that of normal rats (75.58 ± 0.169 nmol/mg). In comparison to diabetic rats, the GSH levels of STZ-induced diabetic rats treated orally with 2.5 mg/kg and 5 mg/kg of Nicorandil were reported to be 54.57 ± 0.1095 µmol/min/g and 59.30 ± 0.0931 µmol/min/g, respectively.

Table 15 : Effect of treatment with nicorandil for six weeks on GSH levels in kidney homogenate in STZ induced diabetic rat

Sl.no	Groups	GSH levels (nmol/mg protein) in kidney homogenate
01	Normal control	75.58 ± 0.1693
02	Diabetic control	43.83 ± 0.2152$^{###}$
03	Diabetic +Nicorandil (2.5mg/kg)	54.57 ± 0.1095***
04	Diabetic + Nicorandil (5 mg/kg)	59.30 ± 0.0931***

4.5 Histopathology of kidney.

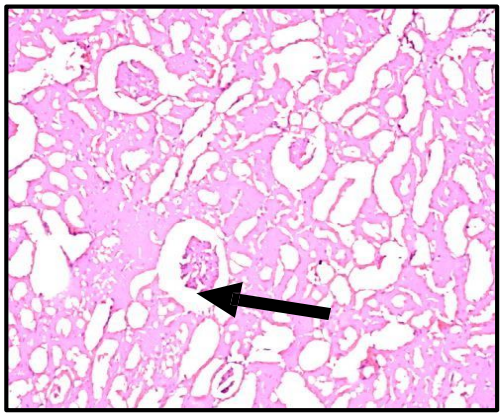

Figure 2 : Section of H& E stained kidney of normal rat under 100X shows normal structure of renal glomeruli and tubules. No Areas of hemorrhage or necrosis

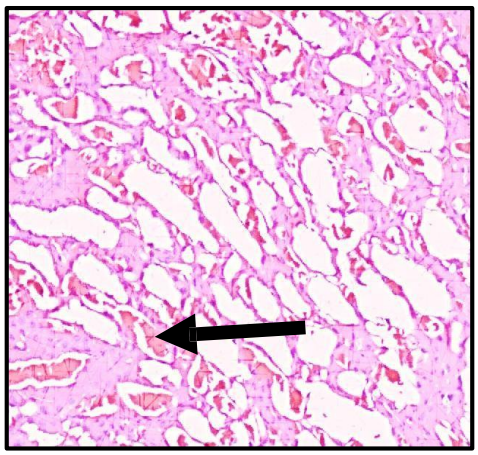

Figure 3 : Section of H& E stained kidney of diabetic rat under 100X shows diffused mesangial cell expansion aggregates of inflammatory cells. Large areas of hemorrhage seen.

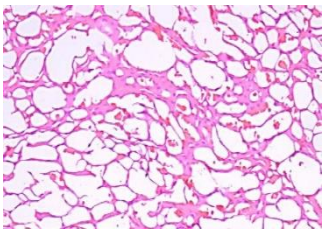

Figure 4: Section of H& E stained kidney of diabetic rat under 100X treated with nicorandil 2.5mg/kg shows decreased mesangial volume Areas of hemorrhage seen. Few areas show hyaline change in tubule

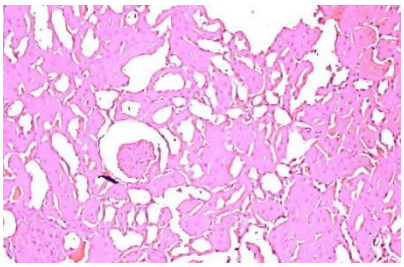

Figure 5 : Section of H& E stained kidney of diabetic rat under 100X treated with nicorandil 5mg/kg shows normal structure of renal glomeruli and tubules. No Areas of hemorrhage or necrosis seen.

DISCUSSION

Streptozotocin IP (52 mg/kg body weight) was used to induce diabetes in order to standardise the dosage; however, the dose was subsequently lowered to 50 mg/kg body weight in order to prevent the death of the

chosen experimental animals. The body weight of diabetic rats was much lower than that of normal rats because of hyperglycemia, hypoinsulinemia, increased muscular atrophy, and tissue protein loss.

Nicorandil was utilised in the current investigation to treat diabetic nephropathy. When compared to normal rats, diabetic rats demonstrated a significant reduction in body weight, urine creatinine levels, creatinine clearance rate, total protein levels, serum albumin levels, and serum total protein levels. Antioxidants such as catalase, GSH, and SOD levels were also significantly reduced. When compared to normal rats, there was a significant rise in the percentage change in blood glucose, renal index, glycosylated haemoglobin levels, urine albumin levels, urinary albumin excretion rate, serum creatinine levels, blood urea nitrogen, and MDA levels.When compared to diabetic rats, insulin-treated diabetic rats demonstrated a significant improvement in urinary creatinine, creatinine clearance rate, serum albumin levels, total protein levels, catalase activity, GSH, and SOD levels. Additionally, there was a significant decrease in body weight, kidney index, blood glucose changes, glycosylated haemoglobin, urinary albumin, urinary albumin excretion rate, serum creatinine, blood urea nitrogen, and MDA levels. According to the current findings, nicorandil has a nephroprotective effect. Unlike other research, nicorandil directly decreased oxidative stress in podocytes through the ATP-dependent K channel, independent of NO, to slow the course of advanced diabetic nephropathy.

Nicorandil stopped the blood glucose level from rising, which was caused by the induction of diabetes. It showed how some medications enhance the effects of insulin by either raising the amount of insulin secreted by the pancreas or by stimulating the beta cells' ability to produce insulin. The protective effect of phloroglucinol against unfavourable protein changes in streptozotocin-induced diabetic rats was documented in previous investigations. As a measure of oxidative stress brought on by diabetes, we have examined the level of GHb. According to our findings, diabetic rats have greater levels of GHb than normal rats. The present investigation unequivocally demonstrated that elevated protein metabolism is confirmed by the decreased blood levels in diabetic rats. We discovered that nicorandil therapy increased serum albumin levels in a dose-dependent way when compared to the diabetes control group. It also inhibited the higher serum creatinine levels in the nicorandil treated groups when compared to the diabetic groups. In our investigation, DN was effectively formed in STZ-induced diabetic rats, as shown by elevated blood levels of metabolic wastes and albuminuria. In DN, serum creatinine is thought to be a sign of a changed glomerular filtration rate (GFR). Reduced tubular reabsorption of filtered plasma proteins has been proposed as the mechanism underlying higher excretion of low molecular weight proteins. Plasma proteins find their way into the ultrafiltrate due to increased glomerular permeability. The current study examined the significantly higher serum total protein levels in the nicorandil-treated group when compared to the diabetic control group, as well as the significantly lower serum total protein levels in the diabetic control group when compared to the normal control group. A recent study found that streptozotocin-induced diabetic kidney impairment in rats is attenuated by a considerable reduction in the blood total protein level in diabetes as compared to normal in response to melatonin and/or rowatinex.

Our recent study's analysis showed that giving the diabetic rats nicorandil therapy greatly reduced their risk of developing microalbuminuria. In the current investigation, urine albumin levels were considerably higher in the diabetic control group relative to the normal control group and significantly lower in the nicorandil-treated group relative to the diabetic control group. The diabetic control group's urinary creatinine was much lower than that of the normal control group, and it was significantly higher in the nicorandil treatment group than in the diabetic control group. Based on earlier research, our findings demonstrated comparable outcomes in rats with diabetic nephropathy produced by STZ. Compared to diabetic control rats, nicorandil treatment decreased the increased urine albumin excretion rate. The diabetes control group's creatinine clearance was much lower. Antioxidant therapy protects the beta-cell from oxidative stress-induced apoptosis and maintains beta-cell activity. Results from previous research indicate that antioxidants improve insulin sensitivity and reduce complications associated with diabetes. As a result, the estimation of oxidants and anti-oxidants is a crucial study parameter. Antioxidants are compounds that have the ability to prevent or delay other molecules from oxidizing. The antioxidant levels in the kidney homogenates were assessed. The results of our current investigation showed that the levels of SOD and GSH were significantly lower in the diabetic control group when compared to the normal control group, and significantly higher in the groups that received nicorandil treatment when compared to the diabetic control group. When compared to the normal control group, the diabetes control group's kidney homogenate's MDA level was much higher, and it was significantly lower.The histopathological studies of sections of kidneys stained with Haematoxylin and Eosin of diabetic rat showed diffused mesangial cell expansion aggregates of inflammatory cells and large areas of haemorrhage was seen. The treatment groups showed reduced inflammation and haemorrhage. The histopathological studies provided the supportive evidence of the nephroprotective activity of nicorandil.Hence nicorandil may be a promising candidate for the protection of kidney damage in diabetes by restoring the biochemical alterations and

modulation of oxidative stress.

CONCLUSION:

Nicorandil's pharmacological assessment for nephroprotection in the rat model of STZ-induced diabetes was the focus of the current investigation. By showing a notable improvement in body weight, urine creatinine, creatinine clearance rate, serum albumin, serum total protein, and antioxidant levels such as catalase, GSH, and SOD in the kidney homogenate, nizoral (2.5 mg/kg) and (5 mg/kg) has been shown to display nephroprotection. When compared to diabetic rats, the treatment groups also shown a substantial decrease in the percentage change in the following parameters: blood glucose, kidney index, glycosylated haemoglobin, urine albumin, urinary albumin excretion rate, serum creatinine, blood urea nitrogen, and MDA levels.By specifically destroying the islets of Langerhans, streptozotocin causes diabetic nephropathy. This leads to the onset of insulin deficiency and chronic hyperglycemia. Nicorandil, our medication, has been shown to have a protective effect against this condition. This effect may be attributed to a number of mechanisms, including the attenuation of oxidative stress and the manifestation of an anti-hyperglycemic effect.
Based on the aforementioned factors, we have determined that the medication Nicorandil, which we have chosen for therapy, demonstrates a noteworthy (***P^{c}0.001) nephroprotective impact. This suggests that Nicorandil may be a useful therapeutic option for halting the advancement of diabetic nephropathy caused by streptozotocin.

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