Effect of Coadministration of Telmisartan with Resveratrol in Streptozotocin-induced Diabetic Nephropathy in Experimental Animals

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Abstract

Aim: to evaluate the effect of a combination of telmisartan and resveratrol on streptozotocin-induced diabetic nephropathy in rats.

Method: A single intraperitoneal injection of streptozotocin (STZ) at a dosage of 55 mg/kg body weight was administered to induce diabetes, using a freshly prepared 0.1 M cold citrate buffer at pH 4.5 as the solvent. Three days post-injection of STZ, diabetes was confirmed through the assessment of serum glucose levels in rats. Rats exhibiting blood glucose levels exceeding 250 mg/dl were classified as diabetic and included in the study protocol. The impact of telmisartan combined with resveratrol on streptozocin-induced diabetic nephropathy in rats was assessed through biochemical parameters, oxidative parameters, and histopathological analysis. Upon concluding the study, the animals were euthanized, and the kidneys were extracted. Parameters of oxidative stress, including GSH, LPO, NO, sodium, and potassium, were assessed, along with a histopathological examination.

Results:

Keywords: Streptozotocin, serum proteins, Diabetes mellitus (DM), oxidative stress, Diabetic nephropathy

1 Introduction:

Diabetes mellitus is fundamentally a chronic condition characterized by persistently elevated blood sugar levels due to issues with insulin function or production in the body. Type 2 diabetes mellitus (T2DM) represents approximately 90% of all diabetes cases, with a significant increase in global incidence from 4.7% in 1980 to 8.5% in 2014 (Ogurtsova et al., 2017). Projections from the International Diabetes Federation (IDF) indicate that there were 77 million individuals with diabetes in India in 2019, with expectations for that figure to rise to 134 million by 2045 (Barman et al., 2023; Saeedi et al., 2019). Insulin production becomes irregular, and the body's response to insulin is also compromised. Additionally, approximately one-third of individuals with diabetes progress to develop diabetic nephropathy (DN), which constitutes a significant kidney complication. (Samsu et al., 2021). Diabetic nephropathy stands out as one of the most common, perilous, and expensive complications associated with diabetes (Alicic et al., 2021). This condition is a progressive, chronic illness that may result in renal failure and currently serves as the primary cause for kidney replacement therapy worldwide (DeFronzo et al., 2021). The development and advancement of diabetic neuropathy are linked to multiple factors, including inflammation, oxidative stress, and hyperglycemia (Singh et al., 2011; DeFronzo et al., 2021). Studies from the literature suggest that in diabetic rats induced with streptozotocin (STZ), inflammation and oxidative stress could lead to direct kidney damage, ultimately resulting in diabetic nephropathy (Barman et al., 2018; Hu et al., 2022). Under hyperglycemic conditions, increased oxidative stress activates pathways such as the P38-MAPK, AKT, and Rheb pathways, leading to tissue fibrosis and inflammation in the kidneys, which ultimately play a role in the progression of diabetic nephropathy (DN) (Fakhruddin et al., 2017; Platé et al., 2020). Studies indicate that anti-oxidative treatment is effective in reducing and delaying the markers and symptoms associated with streptozotocin-induced diabetic neuropathy in rat models (Mahmoodnia et al., 2017). TNF-α, interleukin-1β, and interleukin-6 serve as examples of inflammatory cytokines generated in the kidney following the activation of transcription factors. This process leads to inflammation and dysfunction of the kidneys (King et al., 2008; Mulay et al., 2010).

Telmisartan (TS) exhibits partial agonistic properties towards the "peroxisome proliferator-activated receptor gamma (PPARc)" while also possessing AT1 receptor blocking capabilities. This is utilized in the treatment and management of essential hypertension (Lakshmanan *et al.,* 2011; Ahad *et al.,* 2018). It possesses greater lipophilicity compared to other ARBs, facilitating enhanced tissue and cellular permeation and rendering it more suitable for oral administration (Ayza *et al.,* 2020). Moreover, telmisartan exhibits antioxidative, anti-inflammatory, and anti-proliferative effects in the context of atherosclerosis, underscoring its potential as a valuable pharmacological option for individuals with diabetes who have experienced myocardial infarction (Yamagishi *et al.,*2005; Goyal *et al*., 2011). Trans-3,5,4'-trihydroxystilbene, commonly known as resveratrol (RSV), is a naturally occurring polyphenolic compound present in various foods and beverages, including red wine (Rajasekaran *et al.,* 2011). Due to its potential therapeutic benefits in diabetic heart failure and kidney protection, it has garnered increased interest in DN-related research (Qiao et al., 2017; Yonamine et al., 2016). It was found that by increasing superoxide dismutase activity, RSV reduced oxidative stress in rats with type 2 diabetes (Asadi et al., 2017; Wang *et al.,* 2020). These include its antioxidative properties (Zeng et al., 2021), anti-inflammatory (Xu et al., 2014), cardioprotective (Mokni et al., 2013), neuroprotective (Liu et al., 2015), antihypertensive (Zhang et al., 2021), and blood glucose-lowering (Sadi *et al.,* 2014). Previous studies have demonstrated that resveratrol exhibits renal protective properties in animals with diabetic nephropathy (DN) (Yuan *et al.,* 2018).

The combination of resveratrol and telmisartan has not been previously used in the treatment of diabetes. Therefore, we have decided to use this combination for treating diabetic nephropathy in streptozotocin-induced diabetic rats. This study aimed to evaluate the effect of combining resveratrol with telmisartan on the onset and progression of diabetic nephropathy in animals with experimentally induced diabetes.

2. Materials and methods

2.1Experimental Animals:

For the study, healthy male Wistar rats, weighing between 250 and 300 grams, were selected. The animals were obtained from the K.B.H.S.S. Institute of Pharmacy, situated in Malegaon, Nashik. The animals were maintained in polypropylene cages with adequate ventilation under regulated conditions, which included a temperature of 25 ± 2°C and a 12-hour light/dark cycle. The subjects received consistent pelletized feed from Nutrivet Life Science in Pune, along with access to clean water. The study protocol underwent a thorough review and received approval from the institutional animal ethics committee, confirming adherence to the relevant ethical standards and guidelines. (IAEC No. KBH/2023/-02).

2.2 Drugs and Chemicals:

Streptozotocin (Ottochemie Laboratories, Mumbai, India), Telmisartan (Macleod's Laboratories, Bhilad, Gujarat, India), Resveratrol (Sami Laboratories, Bangalore), Biochemical Estimation Kits (Meril Diagnostics Pvt. Ltd, Gujarat, India). All reagents used in the study were of high analytical grade. Telmisartan and Resveratrol were prepared by suspending them in a 1% w/v sodium carboxymethyl cellulose solution. Streptozotocin (STZ) was dissolved in freshly prepared, ice-chilled citrate buffer with a pH of 4.4. The doses for Telmisartan (5 mg/kg and 10 mg/kg) and Resveratrol (5 mg/kg) were selected based on findings from previous studies.

2.3 Experimental designs:

Following a one-week acclimation period, the rats were randomly allocated into seven groups, with each group comprising six animals (n = 6). The groups underwent a series of drug treatments over a period of 45 days. The initial cohort, designated as the normal control group, received an oral dose of the vehicle (1% sodium carboxymethyl cellulose, Na-CMC) only. The second group, designated as the positive control group, was administered 1% Na-CMC at a dosage of 1 mL/kg via oral delivery. Conducted daily over a period of 45 days. The third and fourth groups, referred to as the standard group, received telmisartan at doses of 5 mg/kg and 10 mg/kg, respectively, administered orally in a 1% sodium carboxymethylcellulose (Na-CMC) solution once daily for a period of 45 days. The fifth group of animals was administered resveratrol (5 mg/kg in 1% Na CMC in d.w., p.o.) daily from day 1 through day 45. A cohort of 6th and 7th animals received telmisartan (5 mg/kg in 1% Na CMC in d.w., p.o.) in conjunction with resveratrol (5 mg/kg in 1% Na CMC in d.w., p.o.) Telmisartan (10 mg/kg in 1% Na CMC in d.w., p.o.) and resveratrol (5 mg/kg in 1% Na CMC in d.w., p.o.) were given once daily for a period of 45 days. Body weight was recorded on the first and forty-fifth days of the treatment, along with measurements of serum glucose (GOD/POD Method), serum triglycerides, serum total cholesterol, serum HDL cholesterol, serum albumin, serum creatinine, serum total protein, blood urea nitrogen, and HbA1c on those same days. The evaluation of antioxidant enzyme assays was performed following the conclusion of the experimental period.
Upon completion of the treatment, the animals will be euthanized using an overdose of urethane. Subsequently, kidney samples will be carefully dissected and rinsed in ice-cold saline. A kidney sample from each group was quickly preserved in a 10% formalin solution for histopathological analysis. The remaining samples were employed for the assessment of oxidative parameters.

2.4 Preparation of STZ solutions

Streptozotocin (STZ) was dissolved in a freshly prepared citrate buffer, maintained at an ice-chilled temperature, with a pH of 4.4. All drug solutions were freshly prepared before administration.

2.5 Induction of diabetes

A single intraperitoneal injection of streptozotocin induced diabetes, (STZ) (55 mg/kg body weight) dissolved in freshly prepared 0.1 M cold citrate buffer (pH 4.5). Diabetes was confirmed 3 days after the injection of STZ by estimating the serum glucose level of rats. Rats with blood glucose levels higher than 250 mg/dL were considered diabetic and were included in the study protocol.

2.6 Biochemical parameters from blood:

On the 1st and 45th days of treatment, all animals were anesthetized using ether as an anesthetic, and blood was collected by puncturing the retro-orbital plexus with a fine glass capillary, then collected in Eppendorf tubes. Serum was isolated through centrifugation and subsequently utilized for the assessment of serum glucose (GOD/POD Method), serum triglycerides, total serum cholesterol, HDL cholesterol, serum albumin, serum creatinine, total serum protein, blood urea nitrogen, and HbA1c, employing the Meril diagnostic kit from India.

2.7 Study of morphometric parameters:

Body weight measurements were taken on the first day and the forty-fifth day of the treatment period. At the conclusion of the study, the kidney was isolated and weighed.

2.8 Antioxidant enzymes assay

At the conclusion of the experimental period, the remaining four animals from each group were euthanized using an overdose of urethane. The kidney was excised and weighed. Kidney homogenates (5% w/v) were prepared in a cold 30 mM Tris buffer (pH 7.4) using a Remi homogenizer to ensure the formation of a clear homogenate. The intact cells and cellular debris were eliminated through centrifugation at 3000 rpm for 10 minutes utilizing a Remi refrigerated centrifuge. The supernatant was used for the assessment of GSH, lipid peroxidation (LPO), Na+/K+ balance, NO, IL-1, IL-6, and TNF-α.

2.9 Assessment of Tissue parameters

2.9.1 Assay of Lipid Peroxidation (LPO):

2.0 mL of the tissue homogenate (supernatant) was combined with 2.0 mL of freshly prepared 10% w/v Trichloroacetic acid (TCA), and the resulting mixture was allowed to rest in an ice bath for 15 minutes. Following a 15-minute interval, the precipitate was isolated by centrifugation, and 2.0 mL of the clear supernatant was combined with 2.0 mL of freshly prepared Thiobarbituric acid (TBA). The solution obtained was heated in a boiling water bath for 10 minutes. It The sample was promptly subjected to an ice bath for a duration of 5 minutes. The developed color was quantified at 532 nm in comparison to a reagent blank. Various concentrations (0-23 nM) of standard malondialdehyde were utilized and processed as previously described for the standard graph. The values were quantified as nMol of MDA per mg of tissue.

2.9.2 Assay of Reduced Glutathione (GSH):

Equal volumes of tissue homogenate (supernatant) and 20% TCA were combined. The precipitated fraction underwent centrifugation, followed by the addition of 0.25 ml of supernatant to 2 ml of DTNB reagent. The final volume was adjusted to 3 ml using phosphate buffer. The developed colour was measured at 412 nm in comparison to the reagent blank. Various concentrations ranging from 10 to 50 µg of standard glutathione were utilized, and the samples were processed as previously described for

the standard graph. The quantity of reduced glutathione was quantified as µg of GSH/mg protein (Moron 1997).

2.9.3 Assay of Nitric oxide (NO):

To 1 mL of tissue homogenate, 1 mL of Griess reagent was added and incubated for 15 minutes at 37°C. Measure the absorbance at 540nm using a Griess reagent blank as a reference. A sodium nitrite solution served as the standard for this study. The concentration of nitrite in the samples was determined using standard curves generated.

Standard curve:

Measure 0, 5, 10, 20, and 50 mL of nitrite standard (corresponding to 0, 2.5, 5, 10, and 25 µg of nitrite) into 100 mL volumetric flasks, and then dilute to approximately 80 mL with water. Introduce 10 mL of sulphanilamide solution into each flask and ensure thorough mixing. Following a 3-minute interval, introduce 1 mL of the coupling reagent, bring the volume to the mark with water, ensure thorough mixing, and allow it to stand for 15 minutes. Determine the absorbance of the solutions relative to water at 540 nm utilizing 10 mm cuvettes. Construct a standard curve plotting absorbance against the concentration of nitrite, ensuring the result is a linear relationship.

Sample:

Precisely measure approximately 1g of the sample, ensuring accuracy to the nearest 0.001g. Combine with water and adjust the volume to 100 ml. Transfer 20 mL into a 100 mL volumetric flask and dilute to approximately 80 mL with water. Introduce 10 ml of sulphanilamide solution and ensure thorough mixing. 3. Following a 3-minute interval, introduce 1 ml of the coupling reagent, dilute to the specified mark with water, mix thoroughly, and allow the solution to stand for 15 minutes. 4. Assess the absorbance of the solution in comparison to water at 540 nm utilizing 10 mm cuvettes. 5. Consult the standard curve to determine the nitrite concentration that corresponds to the measured absorbance.

2.10 Pro-inflammatory cytokinins:

The serum was sent to Shree Bios Innovation Laboratory, Pune, India, for cytokine level detection, and the analysis was performed according to the manufacturer's protocol.

2.11 Histopathological examination:

The kidney tissue was excised from the rats promptly following their sacrifice for histological analysis. One kidney specimen was fixed in 10% phosphate-buffered formalin, dehydrated in a graded series of alcohols, and embedded in paraffin for sectioning. The kidney samples were subsequently dispatched to the Shree Bios Innovation Laboratory located in Pune, India.

2.12 Statistical analysis:

A one-way analysis of variance (ANOVA) was utilized for statistical analysis, accompanied by post hoc Bonferroni’s multiple comparison for hemodynamic parameters. The analysis of data for each parameter was conducted using one-way ANOVA, followed by Dunnett’s post hoc test, utilizing GraphPad Prism software, version 8.2, USA.

3 Results:

3.1 Effect of telmisartan and resveratrol on serum glucose in STZ-induced diabetes in rats.

The diabetic control group exhibited a notable elevation in serum glucose levels compared to the normal control group (###$p<0.001$), indicating pronounced hyperglycemia characteristic of diabetes. The administration of telmisartan at doses of 5 (***$p < 0.001$) and 10 (***$p < 0.001$) mg/kg resulted in a significant decrease in blood glucose levels on the 45th day compared to the disease control group. The

elevated dosage of telmisartan demonstrated a marginally greater efficacy compared to the reduced dosage in mitigating hyperglycaemia. The administration of Resveratrol at a dosage of 5 (***p < 0.001) mg/kg resulted in a notable decrease in blood glucose levels on the 45th day, in comparison to the disease control group (Fig. 1). The combination of Telmisartan and Resveratrol (TR1) demonstrated a significant enhancement in serum cholesterol levels when compared to the diabetic control group (***p<0.001). Furthermore, glucose levels in this group were nearly normalized, indicating a notable synergistic effect. The combination of Telmisartan and Resveratrol (TR2) demonstrated the most pronounced decrease in serum glucose levels, approaching normal control levels. The decrease was notable in comparison to the diabetic control group (***p < 0.001).

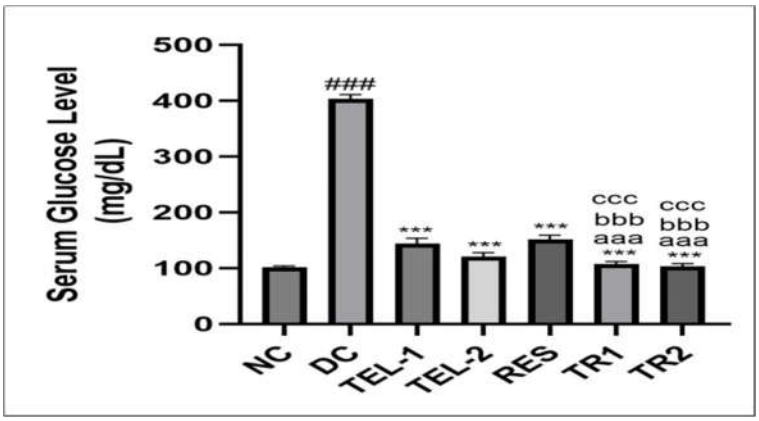


Fig. 1: Effect of telmisartan and resveratrol on serum glucose in STZ-induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

Data are shown as means ± SD (n=6/group). One-Way ANOVA followed by the Bonferroni test for multiple comparisons. ###p<0.001 in comparison to normal control (NC); ***p<0.001 in comparison to diabetic control (DC); aaap<0.001 in comparison to TEL1 group; bbbp<0.001 in comparison to TEL2 group; and cccp<0.001 in comparison to RES group.

3.2 Effect of telmisartan and resveratrol on serum cholesterol in STZ-induced diabetes in rats.

As illustrated in Fig. 2, the serum cholesterol level in the disease control group was significantly elevated at all time points compared to the normal control group (###p < 0.001). The administration of a low dose of telmisartan (5 mg/kg) resulted in a notable decrease in serum cholesterol levels compared to the high dose on the 45th day, when assessed against the disease control group (***p < 0.001) &(***p < 0.001) respectively. The administration of Resveratrol at a dose of 5 (***p < 0.001) mg/kg resulted in a notable decrease in serum cholesterol levels by the 45th day when compared to the disease control group, although the effect was less pronounced than that observed with telmisartan at a dose of 5 mg/kg. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction of serum cholesterol levels when compared to the DC group (***p<0.001). Furthermore, this combination demonstrated highly significant reductions when compared to the TEL-2 (bbbp<0.001) and Resveratrol (cccp<0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable decrease in serum cholesterol levels when compared to the DC group (***p<0.001). The reduction observed was notably significant when compared to the TEL-2 (aaap<0.001) and Resveratrol (cccp<0.001) groups.

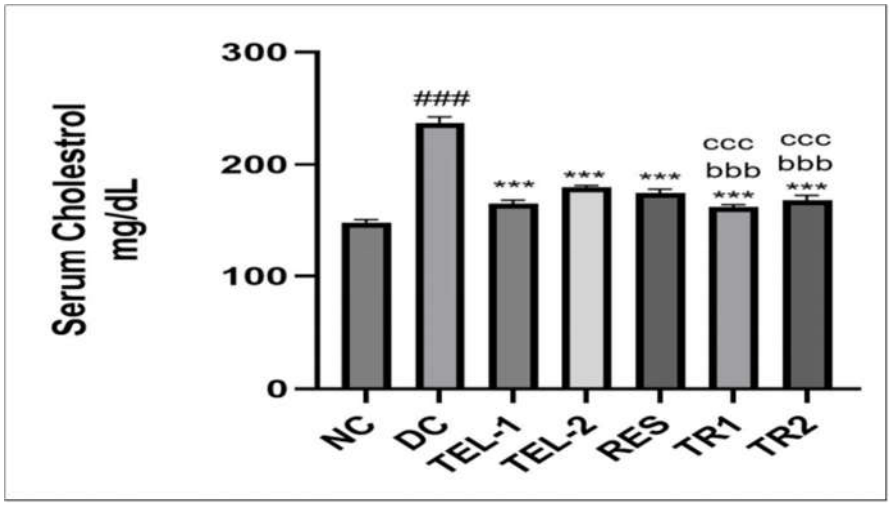


Fig 2: Effect of telmisartan and resveratrol on serum cholesterol in STZ-induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol.

The data are expressed as means ± standard deviation (n=6 per group). One-Way ANOVA followed by Bonferroni test for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); bbbp<0.001 when compared to TEL2 group; and cccp<0.001 when compared to RES group.

3.3 Effect of telmisartan and resveratrol on serum triglyceride in STZ-induced diabetes in rats.

As illustrated in fig-3, the serum triglyceride level in the disease control group was markedly elevated at all points when compared to the normal control group (###p<0.001). The low dose of telmisartan (5 mg/kg) demonstrated a significant reduction in serum triglyceride levels compared to the high dose of telmisartan (10 mg/kg) on the 45th day when analyzed against the disease control group (***p < 0.001) & (***p < 0.001) respectively. The administration of Resveratrol at a dose of 5 (***p < 0.001) mg/kg resulted in a notable decrease in serum triglyceride levels on the 45th day, in comparison to the disease control group. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction in serum triglyceride levels when compared to the DC group (***p<0.001). Furthermore, this combination demonstrated notable reductions when compared to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.001), and RES (cccp<0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable decrease in serum triglyceride levels when compared to the DC group (***p<0.001). The reduction observed was notably significant when compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and it demonstrated a significant superiority over the TEL2 group (bbbp<0.001). The last group, Telmisartan + Resveratrol (TR2), demonstrated a more significant effect compared to the other groups.

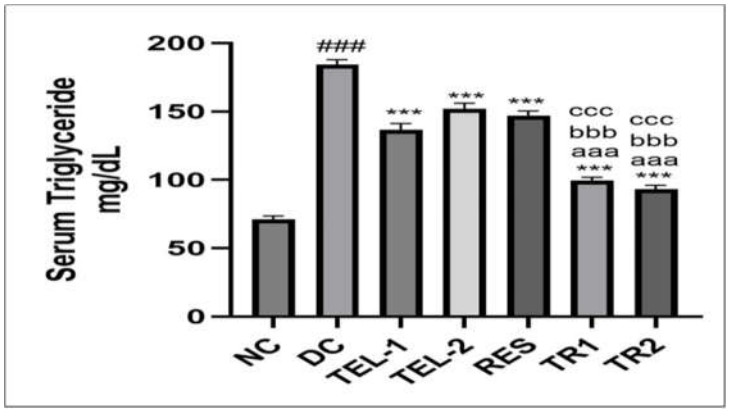


Fig 3: Effect of telmisartan and resveratrol on serum triglyceride in STZ-induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

Data are shown as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni adjustment for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group; bbbp<0.001 when compared to TEL2 group; and cccp<0.001 when compared to RES group.

3.4 Effect of telmisartan and resveratrol on HDL-cholesterol in STZ-induced diabetes in rats

The group with diabetes management exhibited notably lower levels of HDL-cholesterol. This significant reduction (###p<0.001 compared to NC) underscores the detrimental effect of diabetes on lipid metabolism, especially in diminishing protective HDL-cholesterol levels. Treatment with Telmisartan at doses of 5 mg/kg (***P < 0.001) and 10 mg/kg (***P < 0.001) resulted in a significant increase in HDL-cholesterol compared to the disease control group (fig-4). However, when comparing the lower dose of telmisartan to the higher dose, the lower dose demonstrates a more established efficacy than the higher dose. The group treated with resveratrol exhibited HDL-cholesterol levels that were significantly elevated compared to the DC group (***p<0.001 in comparison to DC). This illustrates how resveratrol effectively enhances HDL-cholesterol levels, nearing those observed in the Normal control group. The treatment led to HDL-cholesterol levels that were markedly elevated compared to the DC group (***p<0.001 in relation to DC) and also significantly higher than those observed in the TEL-2 group (***p<0.001 in comparison to TEL-2). The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant increase in HDL-cholesterol levels when compared to the DC group (***p<0.001). Furthermore, this combination demonstrated notable reductions when compared to the TEL2 group (bbbp<0.001) and RES (cccp<0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable enhancement in HDL-cholesterol levels when compared to the DC group (***p<0.001). The reduction observed was notably significant when compared to the RES (cp<0.05) group and demonstrated a significantly greater effect than the TEL2 group (bbbp<0.001). The combination of Telmisartan and Resveratrol (TR1) demonstrates a more significant effect compared to other groups, indicating a greater potential for enhancing lipid profiles.

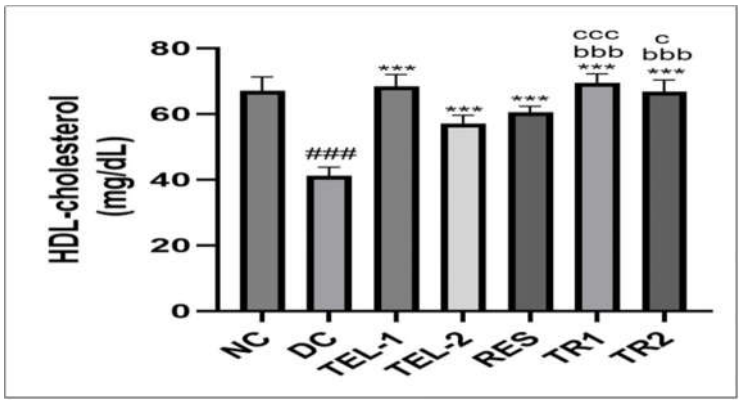


Fig 4: Effect of telmisartan and resveratrol on HDL-cholesterol in STZ induced diabetes in rats

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol.

Data are shown as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni correction for multiple comparisons. ###p<0.001 in comparison to Normal control (NC); ***p<0.001 in relation to diabetic control (DC); ***p<0.001 when contrasted with the disease control group; bbbp<0.001 when assessed against the TEL2 group and cp<0.05 when evaluated against the RES group.

3.5 Effect of telmisartan and Resveratrol on LDL-cholesterol in STZ induced diabetes in rats.

The group with diabetic control exhibited a notable rise in LDL-cholesterol levels when contrasted with the normal control group (###p<0.001). The administration of telmisartan at doses of 5 (***p < 0.001) and 10 (***p < 0.001) mg/kg resulted in a significant decrease in LDL-cholesterol levels on the 45th day, in comparison to the disease control group. The two doses of telmisartan demonstrated a marginally greater efficacy compared to the dose of Resveratrol. The administration of Resveratrol at a dose of 5 mg/kg resulted in a significant reduction in LDL-cholesterol levels on the 45th day, as compared to the disease control group (***p < 0.001). Both combination groups (TR1 and TR2) demonstrated notable decreases in LDL-cholesterol levels when compared to the DC group, with p-values < 0.001 (***p < 0.001). Both combination groups appear to have a more significant effect compared to the single dose of telmisartan and resveratrol. The most significant decrease in LDL-cholesterol levels was noted in the final group, underscoring the greater advantage of utilizing higher doses of this compound (fig-5).

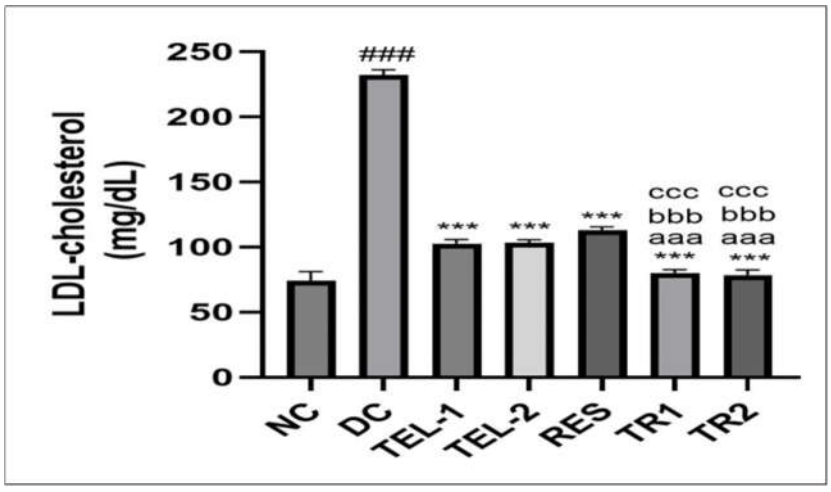


Fig-5 Effect of telmisartan and resveratrol on LDL-cholesterol in STZ induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni adjustment for multiple comparisons. ###p<0.001 in comparison to Normal control (NC); ***p<0.001 in relation to diabetic control (DC); aaap<0.001 relative to TEL1 group; bbbp<0.001 when assessed against TEL2 group and cccp<0.001 when evaluated against RES group.

3. 6 Effect of telmisartan and resveratrol on serum creatinine in STZ induced diabetes in rats.

As demonstrated in fig-6, the serum creatinine level in the disease control group was markedly elevated at all points when compared to the normal control group (###p<0.001). The high dose of telmisartan (10 mg/kg) demonstrated a significant reduction in serum creatinine levels compared to the lower dose of telmisartan (5 mg/kg) in mice on the 45th day, when analyzed against the disease control group (***p < 0.001) & (***p < 0.001) respectively. The administration of Resveratrol at a dosage of 5 (***p < 0.001) mg/kg resulted in a notable decrease in serum creatinine levels on the 45th day, in comparison to the disease control group. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction in serum creatinine levels when compared to the DC group (***p<0.001). Furthermore, this combination demonstrated notable reductions in comparison to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.05), and RES (cccp<0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable decrease in serum creatinine levels when compared to the DC group (***p<0.001). The reduction observed was notably significant when compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and it was also significantly superior to the TEL2 group (bbbp<0.001).

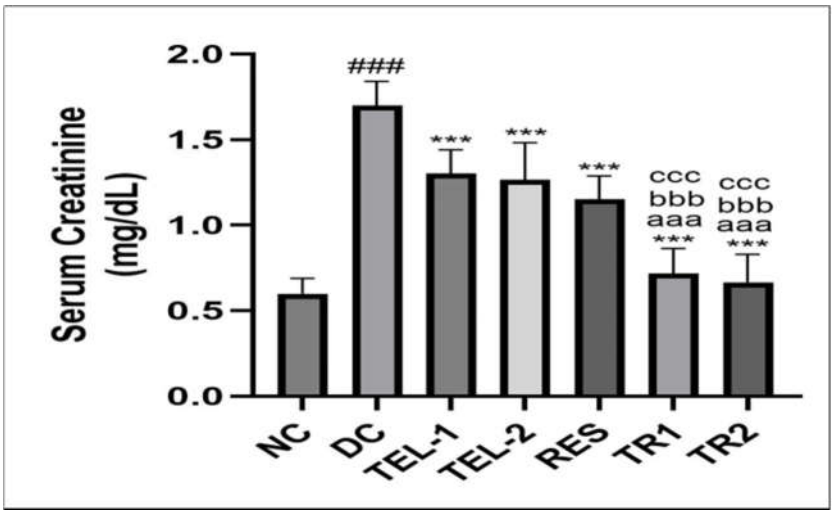


Fig-6 Effect of telmisartan and resveratrol on serum Creatinine in STZ induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni test for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group; bbbp<0.001 when compared to TEL2 group; and cccp<0.001 when compared to RES group.

3.7 Effect of telmisartan and resveratrol on serum BUN in STZ-induced diabetes in rats.

The markedly elevated BUN levels suggest severe kidney impairment due to diabetes. The diabetic control group exhibited a notable rise in serum BUN levels when compared to the normal control group (###p<0.001), indicating pronounced kidney dysfunction characteristic of diabetes. The administration of telmisartan at doses of 5 (***p < 0.001) and 10 (***p < 0.001) mg/kg resulted in a significant decrease in blood BUN levels on the 45th day, in comparison to the disease control group. The elevated dosage of telmisartan demonstrated a marginally greater efficacy compared to the reduced dosage. The administration of Resveratrol at a dose of 5 (***p < 0.001) mg/kg demonstrated a significant reduction in blood serum BUN levels on the 45th day when compared to the disease control group (fig-7). Telmisartan + Resveratrol (TR1): The combined treatment with TEL-1 and resveratrol resulted in a significant improvement in serum BUN levels when compared to the diabetic control group (***p<0.001). The serum BUN levels in this group resembled those of the normal control levels, indicating a notably effective synergistic effect. The combination of Telmisartan and Resveratrol (TR2) demonstrated a notable decrease in serum BUN levels, approaching those of normal control levels. The decrease was noteworthy in comparison to the diabetic control group (***p < 0.001).

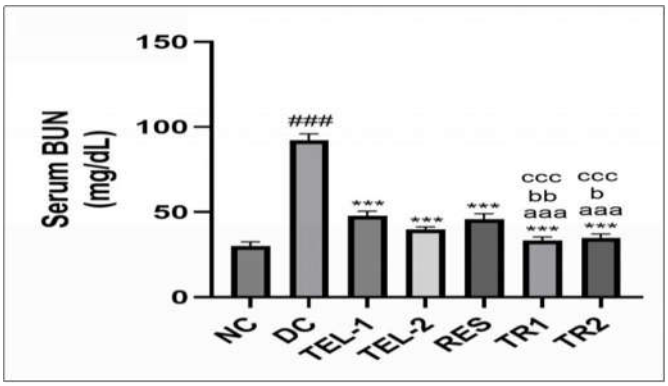


Fig-7 Effect of telmisartan and resveratrol on serum BUN in STZ induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA accompanied by the Bonferroni test for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group; bbp<0.01 & bp<0.05 when compared to TEL2 group and cccp<0.001 when compared to RES group.

3.8 Effect of telmisartan and resveratrol on serum albumin in STZ induced diabetes in rats

The group with diabetic control exhibited notably lower serum albumin levels (###p<0.001 in comparison to NC). The administration of a lower dose of telmisartan in animals resulted in a significant increase in serum albumin levels, demonstrating a notable improvement compared to the DC group (***p < 0.001 compared to the DC group). The higher dose of telmisartan led to serum albumin levels demonstrating an even more pronounced improvement, with a highly significant increase compared to the DC group (***p < 0.001 compared to DC), indicating that it was more effective than the lower dose of telmisartan. The findings indicate a dose-dependent influence of telmisartan on increasing serum albumin levels in diabetic rats. The group treated with resveratrol exhibited significantly elevated serum albumin levels compared to those in the DC group (***p < 0.001). This illustrates the efficacy of Resveratrol in enhancing serum albumin levels, which approach those observed in the normal control group (***p < 0.001). The animal received a low dose combination of telmisartan and resveratrol, which resulted in significantly elevated serum albumin levels compared to the DC group (***p < 0.001), as well as showing significant increases over the TEL-1 group (aaap < 0.01) and the resveratrol group

(cccp < 0.01). The rats that underwent the second novel treatment exhibited a highly significant increase in serum albumin levels compared to the DC group (***p < 0.001). Additionally, there were further significant improvements over both the TEL-1 and TEL-2 groups (aaap<0.001 compared to TEL-1, (bp<0.01 compared to TEL-2) respectively, and the levels were significantly higher than those in the resveratrol group (cccp<0.01 compared to resveratrol), (Fig. 8).

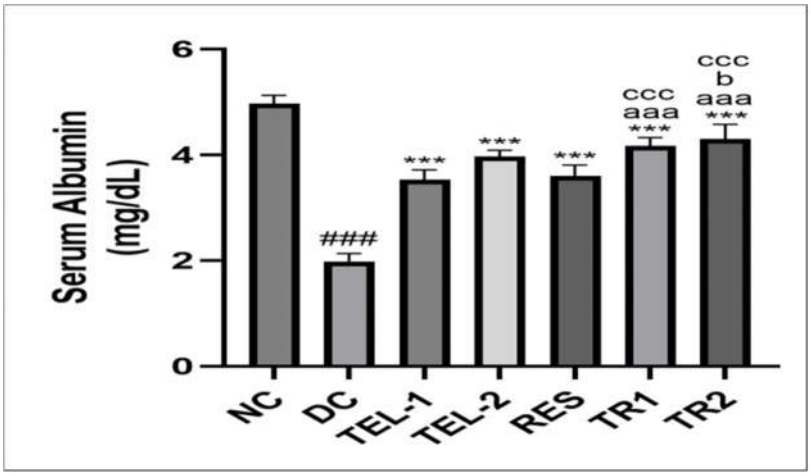


Fig-8 Effect of telmisartan and resveratrol on serum Albumin in STZ induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA accompanied by the Bonferroni test for conducting multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group, bp<0.05 compared to TEL2 group and cccp<0.001 when compared to RES group.

3.9 Effect of telmisartan and resveratrol on serum HbA1c in STZ induced diabetes in rats.

The diabetic control group exhibited a notable elevation in serum HbA1c levels when contrasted with the normal control group (###p<0.001). The administration of telmisartan at doses of 5 (***p < 0.001) and 10 (***p < 0.001) mg/kg resulted in a significant decrease in serum HbA1c levels on the 45th day, in comparison to the disease control group. Both doses of telmisartan demonstrated a marginally greater efficacy than the Resveratrol dose. The administration of Resveratrol at a dosage of 5 (***p < 0.001) mg/kg resulted in a notable decrease in serum HbA1c levels on the 45th day, in comparison to the disease control group. Both combination groups (TR1 and TR2) demonstrated notable decreases in serum HbA1c levels when compared to the DC group (***p < 0.001). Both combination groups appear to have a more significant effect compared to the single dose of telmisartan and Resveratrol. The TR2 group exhibited the most notable decrease in serum HbA1c levels, demonstrating a more significant effect compared to the TEL-1, TEL-2, and RES groups (fig-9).

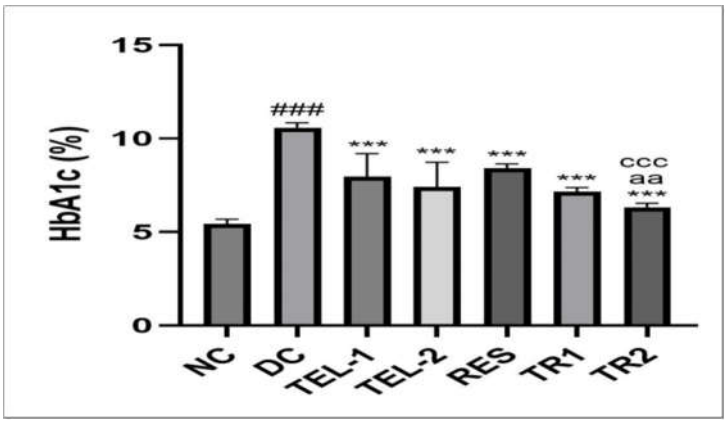


Fig-9 Effect of telmisartan and resveratrol on serum HbA1c in STZ induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by the Bonferroni test for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aap<0.01 compared to TEL1 group and cccp<0.001 when compared to RES group.

3.10 Effect of telmisartan and resveratrol on CRP in STZ-induced diabetes in rats:

As illustrated in Fig. 10, the CRP level in the disease control group was significantly elevated at all points when compared to the normal control group (###p<0.001). The high dose of telmisartan (10 mg/kg) demonstrated a significant reduction in CRP levels compared to the lower dose of telmisartan (5 mg/kg) in mice on the 45th day, when assessed against the disease control group (***p < 0.001) & (***p < 0.001) respectively. The administration of Resveratrol at a dose of 5 (***p < 0.001) mg/kg resulted in a notable decrease in CRP levels on the 45th day, in comparison to the disease control group. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction in CRP levels when compared to the DC group (***p<0.001). Furthermore, this combination demonstrated notable reductions in comparison to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.05), and RES (cccp<0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable decrease in CRP levels when compared to the DC group (***p<0.001). The reduction observed was notably significant when compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and it demonstrated a significant superiority over the TEL2 group (bbbp<0.001).

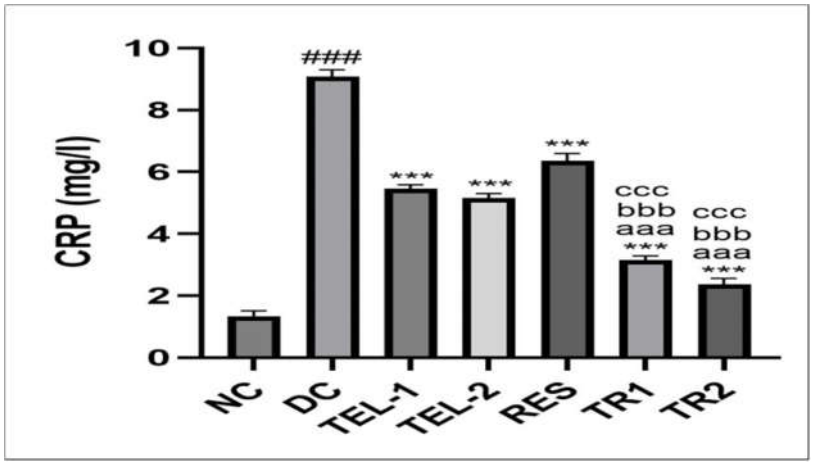


Fig. 10 Effect of telmisartan and resveratrol on CRP in STZ-induced diabetes in rats:

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

Data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by the Bonferroni test for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group; bbbp<0.001 when compared to TEL2 group; cccp<0.001 when compared to RES group.

3.11 Effect of telmisartan and resveratrol on TNF-α in STZ-induced diabetes in rats.

The DC group demonstrates a notable elevation in TNF-α levels relative to the normal control group, suggesting an increase in systemic inflammation linked to diabetes (###p<0.001 when compared to standard control). The administration of telmisartan at a dosage of 5 mg/kg results in a notable decrease in TNF-α levels compared to the diabetic control group (DC). The administration of a higher dose of telmisartan (10 mg/kg) results in a more significant decrease in TNF-α levels compared to the DC and TEL-1 groups. Animals treated with resveratrol at a dosage of 5 mg/kg exhibited a decrease in TNF-α levels when compared to the DC group. The reduction observed is statistically significant (***p < 0.001), suggesting that resveratrol contributes to the reduction of inflammation, albeit with lower efficacy compared to the higher dose of telmisartan. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction in TNF-α levels compared to the DC group (***p < 0.001). Furthermore, this combination demonstrated notable reductions compared to the TEL1 (aaap < 0.001), TEL2 (bbbp < 0.05), and RES (cccp < 0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable decrease in TNF-α levels when compared to the DC group (***p<0.001). The reduction observed was notably significant when compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and it demonstrated a significant superiority over the TEL2 group (bbbp<0.001) (fig-11).

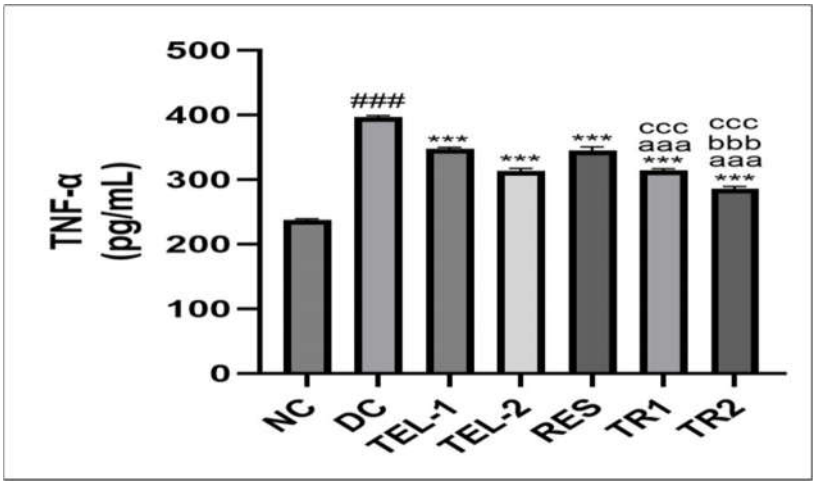


Fig. 11 Effect of telmisartan and resveratrol on TNF-α in STZ-induced diabetes in rats:

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

Data are expressed as means ± SD (n=6/group). One-Way ANOVA accompanied by the Bonferroni test for conducting multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group; bbbp<0.001 when compared to TEL2 group; cccp<0.001 when compared to RES group.

3.12 Effect of telmisartan and resveratrol on IL-1 β in STZ-induced diabetes in rats.

The DC group exhibited a notable increase in IL-1β levels compared to the normal control group, indicating an elevation in systemic inflammation associated with diabetes. The "###" signifies that this increase is statistically significant (###p<0.001) when compared to the normal control group (NC). The administration of telmisartan at doses of 5 (***p < 0.001) and 10 (***p < 0.001) mg/kg resulted in a significant decrease in IL-1β levels on the 45th day compared to the disease control group. Notably, the 10 mg/kg dose of telmisartan demonstrated a marginally greater efficacy than the lower dose and Resveratrol treatment. The administration of Resveratrol at a dosage of 5 mg/kg resulted in a notable decrease in IL-1 β levels (***p < 0.001) on the 45th day, in comparison to the disease control group. Both combination groups (TR1 and TR2) exhibited notable decreases in IL-1β levels compared to the DC group, with p-values of < 0.001 (***p < 0.001). Both combination groups appear to have a more significant effect compared to the single doses of telmisartan and Resveratrol. The most significant decrease in IL-1 β levels was noted in the TR2 group, underscoring the advantages of utilizing elevated doses of this compound (Fig. 12).

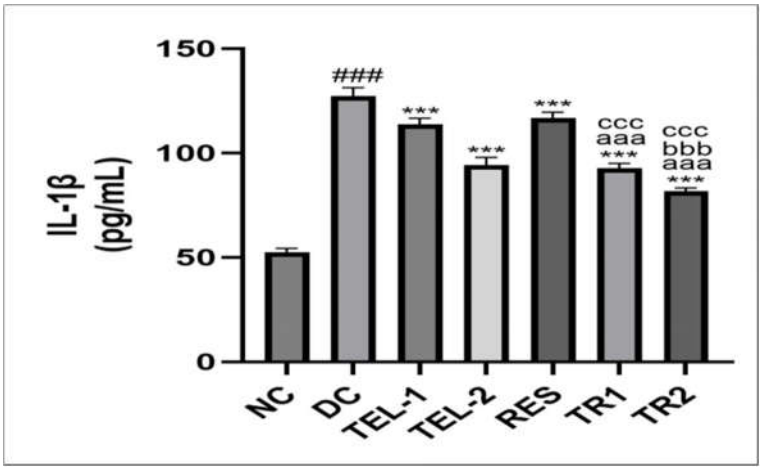


Fig. 12 Effect of telmisartan and resveratrol on IL-1 β in STZ-induced diabetes in rats:

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni correction for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 compared to TEL1 group; bbbp<0.001 when compared to TEL2 group; cccp<0.001 when compared to RES group

3.13 Effect of telmisartan and resveratrol on IL-6 in STZ-induced diabetes in rats.

As shown in Fig. 13, the IL-6 level in the disease control group was significantly elevated at all time points compared to the normal control group (###p < 0.001). The high dose of telmisartan (10 mg/kg) demonstrated a significant reduction in IL-6 levels compared to the lower dose of telmisartan (5 mg/kg) in mice on the 45th day, when assessed against the disease control group (***p < 0.001) & (***p < 0.001) respectively. The administration of Resveratrol at a dose of 5 mg/kg (***p < 0.001) resulted in a significant reduction in IL-6 levels on the 45th day compared to the disease control group, demonstrating effectiveness comparable to that of telmisartan at 5 mg/kg. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction of IL-6 levels when

compared to the DC group (***p<0.001). Furthermore, this combination demonstrated notable reductions in comparison to the TEL1 (aaap<0.001), TEL2 group (cccp<0.001), and RES (cccp<0.001) groups. The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a notable decrease in IL-6 levels when compared to the DC group (***p<0.001). The reduction observed was markedly significant when compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and it demonstrated a significant superiority over the TEL2 group (bbbp<0.001).

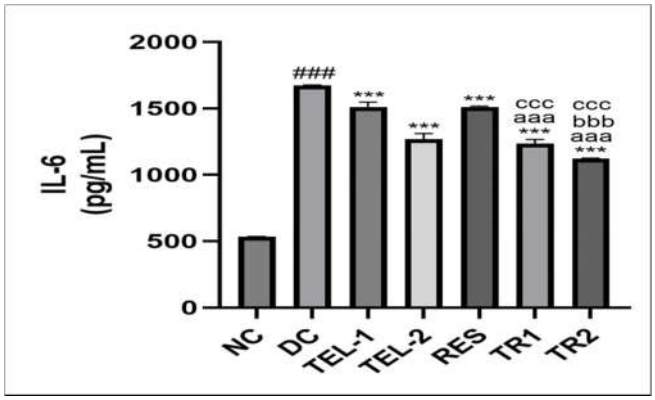


Fig. 13 Effect of telmisartan and resveratrol on IL-6 in STZ-induced diabetes in rats:

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni correction for multiple comparisons. ###p<0.001 in comparison to normal control (NC); ***p<0.001 in comparison to diabetic control (DC); aaap<0.001 in comparison to TEL1 group; bbbp<0.001 in comparison to TEL2 group; and cccp<0.001 in comparison to RES group.

3.14 Impact of telmisartan and resveratrol on body weight (g) in rats with STZ-induced diabetes.

As shown in Fig. 14, the body weights in the disease control group were significantly lower at all points compared to the normal control group (###p<0.001). Treatment with telmisartan at doses of 5 mg/kg and 10 mg/kg resulted in a significant increase in body weights on the 45th day when compared to the disease control group (***p < 0.001) and (***p < 0.001), respectively. The administration of Resveratrol at a dose of 5 mg/kg resulted in a significant increase in body weight on the 45th day compared to the disease control group (***p < 0.001). The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant increase in body weights compared to the DC group (***p<0.001). Furthermore, this combination demonstrated notable increases when compared to the TEL1 (aap<0.01), TEL2 group (bp<0.05), and RES (ccp<0.01) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) led to a significant increase in body weights compared to the DC group (***p<0.001). The increase in weight was found to be highly significant when compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and it was also significantly superior to the TEL2 group (bp<0.05).

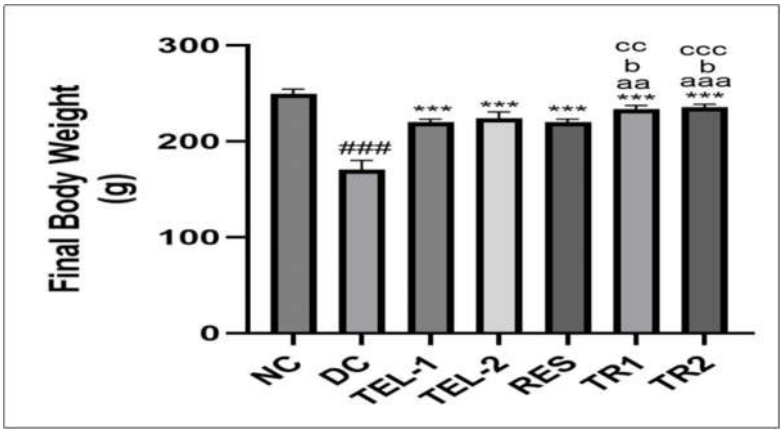


Fig. 14: Effect of telmisartan and resveratrol on body weight (g) in STZ-induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA followed by Bonferroni adjustment for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 and aap<0.01 compared to TEL1 group; bp<0.005 when compared to TEL2 group and cccp<0.001, ccp<0.01 when compared to RES group.

3.15 Impact of telmisartan and resveratrol on renal weight (g) and the kidney hypertrophy index in rats with STZ-induced diabetes.

As shown in Figure 15, the kidney weight in the disease control group increased significantly at all points compared to the normal control group (###p < 0.001). The high dose of telmisartan (10 mg/kg) demonstrated a significant reduction in kidney weight compared to the lower dose of telmisartan (5 mg/kg) in mice on the 45th day, when analyzed against the disease control group (***p < 0.001) & (***p < 0.001) respectively. The administration of Resveratrol at a dose of 5 mg/kg resulted in a significant reduction in kidney weight on the 45th day when compared to the disease control group, demonstrating greater efficacy than telmisartan at 5 mg/kg. The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) resulted in a significant reduction in kidney weight compared to the DC group (***p < 0.001). Furthermore, this combination demonstrated notable reductions compared to the TEL2 group (p < 0.01). The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a notable decrease in kidney weight compared to the DC group (***p < 0.001). The reduction observed was notably significant when compared to the TEL1 (aap<0.01).

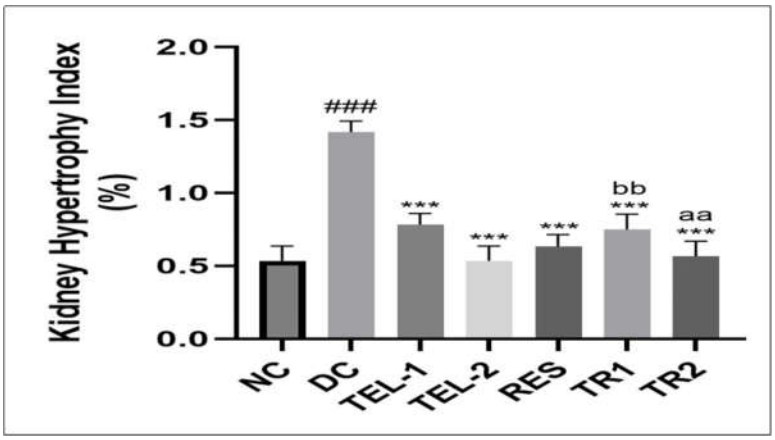


Fig. 15 Effect of telmisartan and resveratrol on kidney weight (g) and kidney hypertrophy index in STZ-induced diabetes in rats

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). One-Way ANOVA accompanied by the Bonferroni test for multiple comparisons. ###p<0.001 when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aap<0.01 compared to TEL1 group; bbp<0.005 when compared to TEL2 group.

3.16 Effect of telmisartan and resveratrol on Antioxidant activities in STZ-induced diabetes in rats

Table 1 reveals that the STZ-induced diabetes group shows a significant increase in LPO and serum K+ levels compared to the normal control group (###P < 0.001), along with a notable decrease in GSH (###P < 0.001), NO (###P < 0.001), and serum Na+. Treatment with Telmisartan at doses of 5 mg/kg and 10 mg/kg for 45 days results in a significant reduction of LPO and serum K+ levels (***P < 0.001 for both), and a significant increase in GSH levels (***P < 0.001 for both) and NO levels (***P < 0.001 for both) when compared to the disease control group. The group administered resveratrol at a dosage of 5 mg/kg for 45 days exhibited a significant reduction in lipid peroxidation (LPO) and serum potassium (K+) levels (***P < 0.001). Additionally, there was an increase in glutathione (GSH) levels, although this was not statistically significant when compared to the telmisartan group (*P < 0.05). Notably, nitric oxide (NO) levels were significantly elevated (***P < 0.001) in comparison to the disease control group, while the combination of Telmisartan (5 mg/kg) and Resveratrol (5 mg/kg) (TR1) was also evaluated. This combined therapy demonstrates a statistically significant enhancement (***p<0.001) compared to the DC group. Combination of Telmisartan and Resveratrol (TR2): The pairing of a higher dosage of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) results in the most pronounced decrease in LPO levels, surpassing all other groups, while also enhancing GSH and NO levels.

Sr no	**Treatment**	**LPO (nmol/mg of protein)**	**GSH (mg/g of protein)**	**NO (nm/g of tissue)**	**Serum Sodium (mmol/L)**	**Serum Potassium (mmol/L)**
1	Normal control	4.65 ± 0.22	27.67 ± 1.50	46.38 ± 1.40	142.3 ± 3.77	4.60 ± 0.28
2	Disease control	13.25 ± 0.18###	14.33 ± 1.36###	27.92 ± 1.52###	104.7 ± 3.77###	8.68 ± 0.27###
3	Telmisartan 5 mg/kg	8.13 ± 1.21***	18.33 ± 1.03***	36.52 ± 1.72***	124.7 ± 6.65***	7.60 ± 0.31***
4	Telmisartan 10 mg/kg	7.71 ± 0.99***	22.83 ± 1.47***	35.73 ± 0.95***	137.2 ± 6.17***	6.75 ± 0.33***
5	Resveratrol 5 mg/kg	8.15 ± 0.64***	13.83 ± 1.47*	42.45 ± 1.48***	120.0 ± 5.21***	7.15 ± 0.28***
6	Telmisartan + Resveratrol (5 + 5 mg/kg)	7.20 ± 0.36***	21.67 ± 1.63***, aa, ccc	51.32 ± 0.76***, aaa, bbb, ccc	134.0 ± 2.82***, a, ccc	6.53 ± 0.36***, aaa, c

7	Telmisartan + Resveratrol (10 + 5 mg/kg)	6.26 ± 0.45***, aaa, bb, ccc	23.67 ± 1.21***, aaa, ccc	55.18 ± 0.78***, aaa, bbb, ccc	136.8 ± 3.81***, aa, ccc	5.95 ± 0.18***, aaa, bbb, ccc

Table 1: Effect of telmisartan and resveratrol on Antioxidant activities

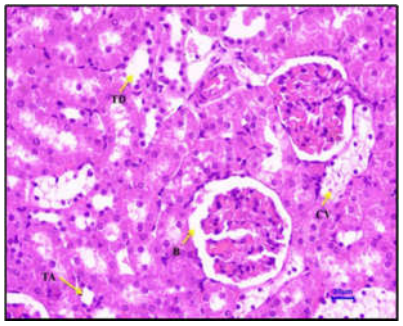
NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

The data are expressed as means ± SD (n=6/group). Utilization of One-Way ANOVA in conjunction with the Bonferroni test for conducting multiple comparisons. ###p<0.001 in comparison to normal control (NC); ***p<0.001, *p<0.05 in comparison to diabetic control (DC); aaap<0.001), aap<0.01 & ap<0.05 in relation to TEL1 group; bbbp<0.001 & bbp<0.01 in relation to TEL2 group and cccp<0.001 in comparison to RES group.

3.17 Impact of telmisartan and resveratrol on kidney histopathology in rats with STZ-induced diabetes

Figure 16 illustrates the impact of telmisartan and resveratrol on histopathological modifications in the kidneys of rats with STZ-induced diabetes. The histopathological examination indicates that the micrograph of the kidney displays alterations such as tubular dilation (TD), tubular atrophy (TA), dilated Bowman's space (B), and cytoplasmic vacuolation (CV).

As a result, from the figure. 16 Kidney histopath, pathological changes in the kidney due to the treatment of STZ-induced DN, which are significantly attenuated by the treatment of telmisartan and resveratrol



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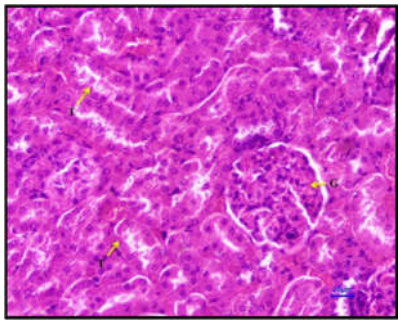
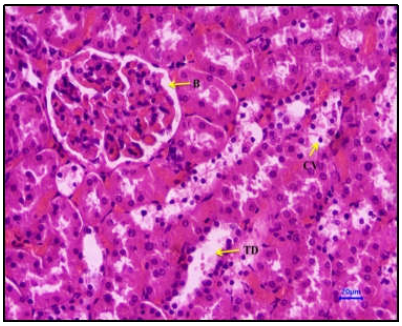
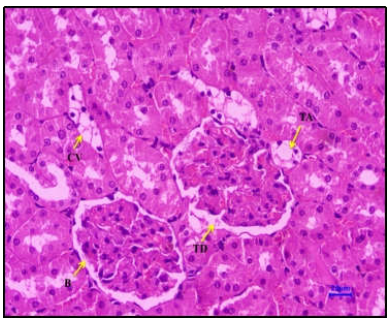


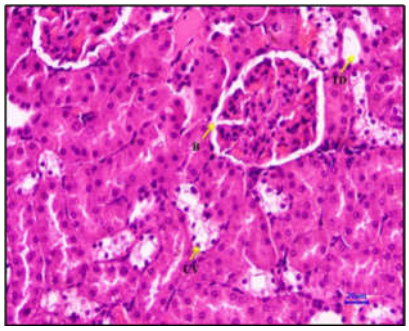
2 Diabetic control



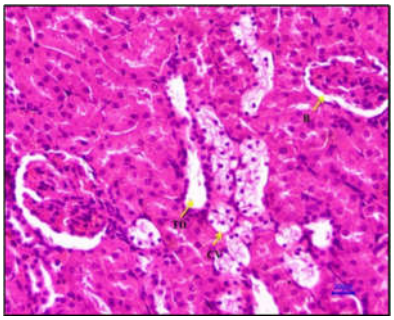
3. Telmisartan- I (5 mg/kg)



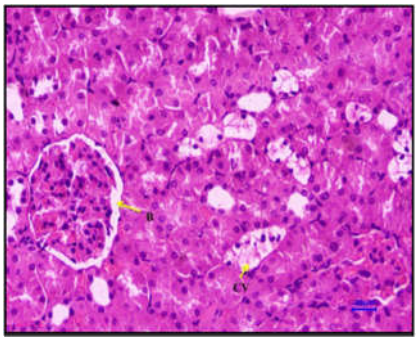
4. Telmisartan- I (10 mg/kg)



5. Resveratrol (5 mg/kg)



6. Telmisartan- I + Resveratrol (5 + 5 mg/kg)



7. Telmisartan- I + Resveratrol (10 + 5 mg/kg)

The examination of kidney tissue revealed normal histology, including renal tubules (T) and glomeruli (G). Observations included tubular dilation (TD), tubular atrophy (TA), dilated Bowman's space (B), and cytoplasmic vacuolation (CV).

Discussion:

With over 100 million cases globally (6% of the global population), diabetes mellitus represents the most prevalent endocrine disorder. It causes an increase or reduction in blood glucose concentrations and is caused by insufficient or inadequate insulin synthesis by the pancreas. Numerous body systems, including blood vessels, the eyes, kidneys, hearts, and nerves, have been proven to be harmed by it.

In animal models, especially rats, streptozotocin (STZ) is frequently used to cause experimental diabetes. STZ poses a risk to the pancreatic beta cells, which are responsible for producing insulin. A decrease in insulin production and the onset of diabetes are the results of STZ's particular targeting and destruction of these beta cells in rats (Hu et al., 2022). The basic mechanism of STZ-induced diabetes involves the destruction of pancreatic beta cells through the inhibition of glucose transporter 2 (GLUT2). The STZ targets pancreatic beta cells, leading to DNA damage through the generation of reactive oxygen species (ROS) and nitric oxide. As a result, the rat eventually experiences beta-cell loss and develops diabetes (Baig et al., 2020).

Glucose is integral to the development and progression of diabetic nephropathy (DN). Oxidative stress, a major contributor to the pathogenesis of diabetic kidney disease and kidney damage, is brought on by persistent hyperglycemia (Liang et al., 2021). Research has demonstrated that regulating blood glucose levels with insulin therapy helps lessen renal fibrosis by preserving the expression of a protein that inhibits the TGF-β1/Smads signaling pathway (Krishan et al., 2017). Additionally, through triggering oxidative stress and blocking the AKT signalling pathway, fluctuating blood glucose levels worsen kidney injury and accelerate its progression. Furthermore, it has been found that reducing oxidative stress and preventing hypoxia by raising HIF-1α levels can help prevent kidney injury by regulating

energy intake and carbohydrate consumption (Mahajan et al., 2020). These results demonstrate the importance of glucose regulation for both DN prevention and management.

Telmisartan, an angiotensin II receptor blocker, exhibits protective effects in diabetic nephropathy by modulating various pathways. Telmisartan mitigates high-glucose-induced damage in renal glomerular endothelial cells by antagonizing the angiotensin II type 1 receptor (AT1R) (Zhan et al., 2021). It also decreases oxidative stress, inflammation, and apoptosis via the Nrf2/HO-1 signaling pathway (Antar et al., 2022). Furthermore, Telmisartan enhances kidney function by influencing mitochondrial oxidative phosphorylation, the peroxisome proliferator-activated receptor (PPAR) signaling pathway, and the slit diaphragm (Wu et al., 2013).

It has been demonstrated that resveratrol, a naturally occurring polyphenol in red grapes and other plant sources, can help lower cholesterol and reduce the risk of diabetic nephropathy. Through the activation of the SIRT1 pathway, resveratrol helps lower cholesterol levels in diabetic nephropathy (Palsamy et al., 2010). According to Gao et al. (2018), the protein SIRT1 is essential for controlling the body's lipid metabolism and cholesterol levels. Resveratrol has the ability to raise SIRT1 activity, which lowers cholesterol by decreasing hepatic synthesis of cholesterol and boosting hepatic breakdown of it (Jung et al., 2014).

Resveratrol is a compound that enhances renal function by lowering blood glucose levels, inflammation, and oxidative stress (Cai et al., 2020). Furthermore, resveratrol exhibits properties that combat fibrosis, inflammation, and oxidative stress, resulting in improvements in renal indicators, including blood urea nitrogen, serum creatinine, and proteinuria (Xian et al., 2020). The administration of resveratrol to animals showed a significant decrease in serum levels of total cholesterol, LDL, and triglycerides. Therefore, employing this mechanism, the combination of telmisartan and resveratrol has the potential to reduce blood glucose levels.

The total protein level is a crucial indicator of kidney function in diabetic nephropathy. In diabetic nephropathy, the kidneys may become less efficient at filtering waste products, including proteins, from the blood. This can lead to an increase in total protein levels in the blood, specifically albumin, as well as serum creatinine and blood urea nitrogen (BUN), which are markers of kidney damage. Elevated total protein levels in diabetic nephropathy can indicate proteinuria, or the presence of excess protein in the urine, which is a sign of kidney damage and dysfunction (Zhang et al., 2016). The present study demonstrated that animals treated with STZ showed significantly increased protein levels, which were ameliorated by using different treatments of telmisartan at 5 and 10 mg/kg. However, Resveratrol treatment significantly decreased total protein levels in the urine of diabetic rats (Chen et al., 2015). Resveratrol demonstrated protective effects by reducing the expression of pro-inflammatory cytokines and oxidative stress markers, which in turn prevented renal tissue damage and protein leakage into the urine. However, using a combination of telmisartan and resveratrol shows a highly significant reduction in protein levels.

Creatinine is a metabolic byproduct generated by muscle tissue during the degradation of creatine phosphate. It is primarily eliminated from the bloodstream by the kidneys and subsequently excreted in urine. Diabetic nephropathy, a progressive kidney disease associated with diabetes, can be accurately assessed through creatinine levels, which serve as indicators of kidney function (Rostambeig et al., 2010). As a result, creatinine levels may increase in the blood, leading to a condition known as elevated serum creatinine levels or impaired kidney function. In the present study, an increased serum creatinine concentration is associated with the induction of oxidative stress and the blocking of the AKT signalling pathway, which is reduced by ameliorating oxidative stress through the treatment of telmisartan and resveratrol. However, the combination of these two shows more effectiveness than single doses.

Blood Urea Nitrogen (BUN) is a commonly used marker for assessing kidney function, as it reflects the levels of urea in the blood that are typically filtered by the kidneys (Kim et al., 2021). In diabetic

nephropathy, there is damage to the kidneys due to high levels of glucose in the blood, leading to an impaired filtration process and an increase in BUN levels. Elevated BUN levels are linked to an increased risk of diabetic nephropathy progression and serve as a prognostic indicator for chronic kidney disease development in diabetes (Kim et al., 2021). In the present study, BUN levels are increased in the disease control group. This leads to oxidative stress and malfunction in glomerular filtration. The current study shows that both high and low doses of telmisartan had positive effects on BUN levels. In diabetic nephropathy, telmisartan helps to mitigate renal damage and enhance renal function by lowering oxidative stress, inflammation, and fibrosis (Kishida et al., 2008). Resveratrol therapy dramatically lowered BUN levels in comparison to diabetic rats. According to research on the effects of the drug on rat diabetic nephropathy, the antioxidant properties of resveratrol can shield the kidneys from oxidative stress-triggered damage (Kim et al., 2011).

There is a strong connection between elevated levels of HbA1c and the onset and advancement of diabetic nephropathy in both animal models and human subjects (Bahadoran et al., 2923). Elevated HbA1c levels were correlated with the severity of renal dysfunction in diabetic rats with nephropathy (Wen et al., 2016). The present study showed that the diabetic control group increased HbA1c levels, which were significantly mitigated by Telmisartan and resveratrol doses. Telmisartan may lower HbA1c levels in diabetic nephropathy in rats by blocking the AT1 receptor, which reduces inflammation and oxidative stress while enhancing insulin sensitivity and lipid profiles (Vanholder et al., 2018). Resveratrol reduces HbA1c levels in diabetic nephropathy by modulating glucose metabolism, enhancing insulin sensitivity, and suppressing inflammation in the kidney (Wu et al., 2017).

The manifestation of kidney injury has been observed to be correlated with CRP. In diabetic rats, elevated CRP levels are associated with increased oxidative stress, inflammation, and tissue damage in the kidneys. Studies indicate that reducing CRP levels in rat models may improve renal function and decrease the severity of diabetic nephropathy (Navarro-González et al., 2011). This study demonstrates that elevated CRP levels in STZ-induced diabetic rats are reduced through the continuous administration of Telmisartan, resveratrol, or a combination of both over 45 days. Telmisartan provides protective effects in diabetic nephropathy through continuous administration by inhibiting the AT1 receptor, resulting in reduced inflammation and fibrosis, as well as enhanced renal function (Xu et al., 2011). Resveratrol acts as an activator of SIRT1, a NAD+-dependent histone deacetylase that regulates numerous cellular processes, including inflammation. Activation of SIRT1 by resveratrol suppresses CRP expression by inhibiting NF-κB acetylation (Sharma et al., 2022).

It is also well established that the consecutive use of STZ leads to increased oxidative stress in the Kidney and other parts of the body. The present study demonstrates that the administration of STZ has resulted in a substantial rise in the level of oxidative stress in the kidneys of rats, indicated by a reduction in the activity of antioxidant enzymes, including GSH, NO, and sodium, alongside an elevation in LPO and potassium levels. Oxidative stress is identified as a significant factor in the development of diabetic nephropathy (DN), a diabetes-related complication marked by kidney damage. This study demonstrates that Telmisartan and resveratrol exhibit antioxidant properties by mitigating oxidative stress and inflammation across multiple tissues, including the kidneys. This may lead to an increase in GSH levels, as GSH is an important antioxidant molecule that helps protect cells from oxidative damage (Wiart et al., 2023). However, the combination of these two drugs has a more pronounced effect than either one alone.

The current study reveals a significant decrease in NO levels in STZ-induced diabetic rats, which was alleviated by telmisartan and resveratrol through continuous administration for 45 days. Nitric oxide (NO) plays a crucial role in the pathogenesis of the disease (Choi et al., 1990). Telmisartan can help decrease oxidative stress in the kidney. Oxidative stress is known to decrease NO bioavailability, so by reducing oxidative stress, telmisartan can help increase NO levels and improve renal function in diabetic nephropathy (Kohan et al., 2014). Resveratrol helps to preserve NO levels in the kidneys and prevent

endothelial dysfunction (Mohammadi et al., 2024). Additionally, resveratrol has been shown to activate Endothelial nitric oxide synthase (eNOS) is the enzyme that facilitates the production of nitric oxide (NO) within the endothelium.

Interleukin-10 (IL-10) is a significant anti-inflammatory cytokine relevant to diabetic nephropathy. IL-10 exhibits significant anti-inflammatory properties, effectively suppressing the synthesis of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), within the renal system. Research indicates that administering IL-10 can reduce renal inflammation and fibrosis in rat models of diabetic nephropathy (Tesch et al., 2008). Telmisartan inhibits the activation of downstream pathways responsible for the production and release of pro-inflammatory cytokines by blocking the angiotensin II type 1 receptor. Telmisartan has been demonstrated to upregulate the expression of anti-inflammatory cytokines, including interleukin-10 (IL-10), thereby counteracting the inflammatory response (Rodriguez-Naranjo et al., 2013). Resveratrol exhibits anti-inflammatory properties by modulating multiple signaling pathways, resulting in a decrease in pro-inflammatory cytokine expression and an increase in anti-inflammatory cytokine expression (Xin et al., 2024). Resveratrol reduces pro-inflammatory cytokine levels, including TNF-α and IL-6, in a rat model of diabetic nephropathy (Gulcubuk et al., 2014).The combined use of telmisartan and resveratrol effectively reduces pro-inflammatory cytokines in diabetic rats.

Diabetic nephropathy (DN) is a major microvascular complication of diabetes mellitus, mostly caused by oxidative stress and inflammation due to persistent hyperglycemia. This study assessed the prophylactic effects of Telmisartan and resveratrol, both alone and in combination, in a streptozotocin (STZ)-induced diabetic rat model. The rationale for selecting these agents is grounded in their established pharmacological properties: Telmisartan, an angiotensin II receptor blocker (ARB), is acknowledged for its renoprotective and anti-inflammatory effects, while resveratrol, a natural polyphenolic compound, exhibits notable antioxidant and anti-diabetic properties.

Diabetic nephropathy (DN) is a significant microvascular consequence of diabetes mellitus, predominantly resulting from chronic hyperglycemia-induced oxidative stress and inflammation. This study evaluated the preventive effects of Telmisartan and resveratrol, both separately and in conjunction, in a streptozotocin (STZ)-induced diabetic rat model. The justification for choosing these agents is based on their recognized pharmacological characteristics: Telmisartan, an angiotensin II receptor blocker (ARB), is recognized for its renoprotective and anti-inflammatory attributes, whereas resveratrol, a natural polyphenolic molecule, demonstrates significant antioxidant and anti-diabetic actions.

The biochemical findings of our investigation indicated that both Telmisartan and resveratrol, when given separately, significantly improved hyperglycemia and normalized disrupted serum indicators associated with kidney function, including serum creatinine, urea, and blood urea nitrogen (BUN). However, the combination treatment, especially at 10 mg/kg of Telmisartan and 5 mg/kg of resveratrol, provided a more pronounced improvement, suggesting a synergistic effect.

The effects of oxidative stress are pivotal in the pathophysiology of diabetic nephropathy. Our results demonstrated that combination therapy significantly decreased malondialdehyde (MDA) levels and notably enhanced the activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), compared to the enhanced antioxidant capacity of the combination treatment, suggesting that the improved antioxidant capacity of the combo treatment may significantly contribute to its nephroprotective benefits.

Histopathological analysis further supported the biochemical and oxidative data. Renal tissues from diabetic rats showed typical pathological changes, including glomerular hypertrophy, mesangial expansion, and tubular degeneration. The administration of Telmisartan and resveratrol, particularly in

conjunction, led to considerable enhancement in renal architecture, signifying successful mitigation of renal injury.

The combination of resveratrol and telmisartan, at 10 + 5 mg/kg, proved to be the most effective treatment. The superior efficacy of this dose can be attributed to the dual mechanism of action—the renin-angiotensin system (RAS) suppression by telmisartan and the antioxidant and anti-inflammatory properties of resveratrol. Notably, telmisartan functions as a partial agonist of PPAR-γ, potentially augmenting insulin sensitivity and providing additional protective benefits to renal tissues.

Overall, the study provides evidence that the combined use of Telmisartan and resveratrol exerts a potent protective effect against diabetic nephropathy by modulating glycemic control, oxidative stress, and histological damage. These findings open up avenues for potential combination therapies that leverage both pharmaceutical and natural agents to manage diabetic complications more effectively.

Conclusion

This study investigates the prophylactic effects of Telmisartan and resveratrol on STZ-induced diabetes in rats, administered in a dose-dependent manner (5 and 10 mg/kg) and individually at 5 mg/kg, as well as in combination (5 + 5 mg/kg and 10 + 5 mg/kg). Based on Biochemical, oxidative, and histopathological examinations, we conclude that the most appropriate combination dose is Telmisartan and resveratrol (10 + 5 mg/kg), indicating an intense treatment for Diabetic nephropathy.

Abbreviations:	Full forms of Abbreviations
NC	Normal control
DC	Disease control
TEL-1	Telmisartan (5 mg/kg)
TEL-2	Telmisartan (10mg/kg)
RES	Resveratrol,
TR1	Telmisartan + Resveratrol
TR2	Telmisartan + Resveratrol
eNOS	oxide synthase
TNF-α	Tumor Necrosis Factor Alpha
STZ	streptozotocin
GSH	Glutathione
NO	nitric oxide
LPO	Lipid peroxidation
DN	diabetic nephropathy
TD	Tubular Dilation
TA	Tubular atrophy
CV	Cytoplasmic vacuolation

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None

Conflicts of interest:

None

Ethics approval:

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC No. KBH/2023/-02).

Disclaimer statements:

Contributors: Dr. Yogesh Suresh Ahire contributed to the conception and supervision of the study, as well as checking, editing, and all revisions of the manuscript. Smruti R. Jadhav contributed to carrying out all the experiments, statistical analysis of the data, and preparing the first draft of the manuscript, including tables and figures. Shekhar D. Jagtap, Swapnil B. Jadhav, and Vinod A. Bairagi contributed to the preparation and implementation of the study design and also contributed to the data analysis.

References:

1. Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., Cavan, D., Shaw, J.E. and Makaroff, L.E., 2017. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes research and clinical practice, 128, pp.40-50.
2. Barman, P., Das, M. and Verma, M., 2023. Epidemiology of type 2 diabetes mellitus and treatment utilization patterns among the elderly from the first wave of Longitudinal Aging study in India (2017-18) using a Heckman selection model. BMC Public Health, 23(1), p.699.
3. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K. and Shaw, J.E., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice*, *157*, p.107843.
4. Samsu, N., 2021. Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. BioMed research international, 2021(1), p.1497449.
5. Alicic, R.Z., Cox, E.J., Neumiller, J.J. and Tuttle, K.R., 2021. Incretin drugs in diabetic kidney disease: biological mechanisms and clinical evidence. Nature Reviews Nephrology, 17(4), pp.227-244.
6. DeFronzo, R.A., Reeves, W.B. and Awad, A.S., 2021. Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. Nature Reviews Nephrology, 17(5), pp.319-334.
7. Singh, D.K., Winocour, P. and Farrington, K., 2011. Oxidative stress in early diabetic nephropathy: fueling the fire. Nature Reviews Endocrinology, 7(3), pp.176-184.
8. Hu, Y., Wang, S.X., Wu, F.Y., Wu, K.J., Shi, R.P., Qin, L.H., Lu, C.F., Wang, S.Q., Wang, F.F. and Zhou, S., 2022. Effects and Mechanism of Ganoderma lucidum Polysaccharides in the Treatment of Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats. BioMed Research International, 2022(1), p.4314415.
9. Fakhruddin, S., Alanazi, W. and Jackson, K.E., 2017. Diabetes-induced reactive oxygen species: mechanism of their generation and role in renal injury. Journal of diabetes research, 2017(1), p.8379327.
10. Platé, M., Guillotin, D., & Chambers, R. C. (2020). The promise of mTOR as a therapeutic target pathway in idiopathic pulmonary fibrosis. European Respiratory Review, 29, 200269.

11. Mahmoodnia, L., Aghadavod, E., Beigrezaei, S. and Rafieian-Kopaei, M., 2017. An update on diabetic kidney disease, oxidative stress and antioxidant agents. Journal of renal injury prevention, 6(2), p.153.
12. King, G.L., 2008. The role of inflammatory cytokines in diabetes and its complications. Journal of periodontology, 79, pp.1527-1534.
13. Mulay, S.R., Gaikwad, A.B. and Tikoo, K., 2010. Combination of aspirin with telmisartan suppresses the augmented TGFβ/smad signaling during the development of streptozotocin-induced type I diabetic nephropathy. Chemico-biological interactions, 185(2), pp.137-142.
14. Lakshmanan, A.P., Watanabe, K., Thandavarayan, R.A., Sari, F.R., Harima, M., Giridharan, V.V., Soetikno, V., Kodama, M. and Aizawa, Y., 2011. Telmisartan attenuates oxidative stress and renal fibrosis in streptozotocin induced diabetic mice with the alteration of angiotensin-(1–7) mas receptor expression associated with its PPAR-γ agonist action. Free radical research, 45(5), pp.575-584.
15. Ahad, A., Raish, M., Ahmad, A., Al-Jenoobi, F.I. and Al-Mohizea, A.M., 2018. Development and biological evaluation of vesicles containing bile salt of telmisartan for the treatment of diabetic nephropathy. Artificial Cells, Nanomedicine, and Biotechnology, 46(sup1), pp.532-539.
16. Ayza, M.A., Zewdie, K.A., Tesfaye, B.A., Gebrekirstos, S.T. and Berhe, D.F., 2020. Anti-diabetic effect of telmisartan through its partial PPARγ-agonistic activity. Diabetes, Metabolic Syndrome and Obesity, pp.3627-3635.
17. Yamagishi, S. and Takeuchi, M., 2005. Telmisartan is a promising cardiometabolic sartan due to its unique PPAR-γ-inducing property. Medical hypotheses, 64(3), pp.476-478.
18. Goyal, S.N., Bharti, S., Bhatia, J., Nag, T.C., Ray, R. and Arya, D.S., 2011. Telmisartan, a dual ARB/partial PPAR-γ agonist, protects myocardium from ischaemic reperfusion injury in experimental diabetes. Diabetes, Obesity and Metabolism, 13(6), pp.533-541.
19. Rajasekaran, D., Elavarasan, J., Sivalingam, M., Ganapathy, E., Kumar, A., Kalpana, K. and Sakthisekaran, D., 2011. Resveratrol interferes with N-nitrosodiethylamine-induced hepatocellular carcinoma at early and advanced stages in male Wistar rats. Molecular medicine reports, 4(6), pp.1211-1217.
20. Qiao, Y.C., Chen, Y.L., Pan, Y.H., Tian, F., Xu, Y., Zhang, X.X. and Zhao, H.L., 2017. The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: A systematic review and meta-analysis. PloS one, 12(4), p.e0176157.
21. Yonamine, C.Y., Pinheiro-Machado, E., Michalani, M.L., Freitas, H.S., Okamoto, M.M., Corrêa-Giannella, M.L. and Machado, U.F., 2016. Resveratrol improves glycemic control in insulin-treated diabetic rats: participation of the hepatic territory. Nutrition & metabolism, 13, pp.1-10.
22. Asadi-Samani, M., Moradi, M.T., Mahmoodnia, L., Alaei, S., Asadi-Samani, F. and Luther, T., 2017. Traditional uses of medicinal plants to prevent and treat diabetes; an updated review of ethnobotanical studies in Iran. Journal of nephropathology, 6(3), p.118.
23. Wang, T., Lu, J., Shi, L., Chen, G., Xu, M., Xu, Y., Su, Q., Mu, Y., Chen, L., Hu, R. and Tang, X., 2020. Association of insulin resistance and β-cell dysfunction with incident diabetes among adults in China: a nationwide, population-based, prospective cohort study. The lancet Diabetes & endocrinology, 8(2), pp.115-124.
24. Zeng, Q., Xiong, Q., Zhou, M., Tian, X., Yue, K., Li, Y., Shu, X. and Ru, Q., 2021. Resveratrol attenuates methamphetamine-induced memory impairment via inhibition of oxidative stress and apoptosis in mice. Journal of Food Biochemistry, 45(2), p.e13622.
25. Xu, C.X., Zhu, H.H. and Zhu, Y.M., 2014. Diabetes and cancer: Associations, mechanisms, and implications for medical practice. World journal of diabetes, 5(3), p.372.
26. Mokni, M., Hamlaoui, S., Karkouch, I., Amri, M., Marzouki, L., Limam, F. and Aouani, E., 2013. Resveratrol provides cardioprotection after ischemia/reperfusion injury via modulation of antioxidant enzyme activities. Iranian journal of pharmaceutical research: IJPR, 12(4), p.867.

27. Liu, W., Li, G., Hölscher, C. and Li, L., 2015. Neuroprotective effects of geniposide on Alzheimer's disease pathology. Reviews in the Neurosciences, 26(4), pp.371-383.
28. Zhang, L.X., Li, C.X., Kakar, M.U., Khan, M.S., Wu, P.F., Amir, R.M., Dai, D.F., Naveed, M., Li, Q.Y., Saeed, M. and Shen, J.Q., 2021. Resveratrol (RV): A pharmacological review and call for further research. Biomedicine & pharmacotherapy, 143, p.112164.
29. Sadi, G., Bozan, D. and Yildiz, H.B., 2014. Redox regulation of antioxidant enzymes: post-translational modulation of catalase and glutathione peroxidase activity by resveratrol in diabetic rat liver. Molecular and Cellular Biochemistry, 393, pp.111-122.
30. Yuan, D., Liu, X.M., Fang, Z., Du, L.L., Chang, J. and Lin, S.H., 2018. Protective effect of resveratrol on kidney in rats with diabetic nephropathy and its effect on endoplasmic reticulum stress. European Review for Medical & Pharmacological Sciences, 22(5).