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

Dr Yogesh Suresh Ahire, Smruti Ramdas Jadhav, Swapnil Bandu Jadhav, Shekhar Dipak Jagtap, Dr Vinod Ashokdas Bairagi.

Abstract

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

Method: Diabetes was induced by a single intraperitoneal injection of streptozotocin, (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 level higher than 250 mg/dl was regarded as diabetic and involved in the study protocol. For the effect of telmisartan with resveratrol on streptozocin induced diabetic nephropathy in rats was evaluated by, Biochemical Parameters, oxidative parameters and histopathological parameter After completion of the study, animals were sacrificed and Kidney was isolated and oxidative stress parameter such as GSH, LPO, NO, Sodium and potassium were analyzed and histopathological examination also carried.

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

1 Introduction:

Diabetes mellitus is basically a chronic condition where blood sugar levels stay high because the body has the same problem with insulin function or production. Type 2 diabetes mellitus (T2DM) accounts for around 90% of all occurrences of diabetes, with a globalincidence of DM rising sharply from 4.7% in 1980 to 8.5% in 2014 (Ogurtsova *et al.,* 2017). According to projections from the International DiabetesFederation (IDF), there were 77 million diabetics in India in 2019 and thatnumber is expected to increase to 134 million by 2045 (Barman *et al.,* 2023; Saeedi *et al.,* 2019). Insulin production surely becomes abnormal, and moreover, insulin action also gets disturbed in the body. Also, about one-third of diabetic patients further develop diabetic nephropathy (DN), which itself becomes a serious kidney complication. (Samsu *et al.,* 2021). One of the most prevalent, dangerous, and costly consequences of diabetes is diabetic nephropathy (Alicic *et al.,* 2021). It is a progressive, chronic illness that can lead to renal failure and is presently the main reason for kidney replacement therapy globally (DeFronzo *et al.,* 2021). The pathogenesis and progression of diabetic neuropathy are attributed to several variables such as inflammation, oxidative stress, and hyperglycemia (Singh *et al.,* 2011; DeFronzo *et al.,* 2021). Research from the literature indicates that in diabetic rats produced with streptozotocin (STZ), inflammation and oxidative stress may cause direct kidney damage that results in diabetic nephropathy (Barman *et al.,* 2018; Hu *et al.,* 2022). In a hyperglycemic condition, elevated oxidative stress sets off pathways like P38-MAPK, AKT, and Rheb, which in turn cause tissue fibrosis and inflammation in the kidneys, ultimately contributing to the development of diabetic nephropathy (DN) (Fakhruddin et al., 2017; Platé et al., 2020). Research has shown 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-ά, interleukin-1β, and interleukin-6 are examples of inflammatory cytokines that are produced in the kidney upon activation of a transcription factor. The inflammation and malfunction of the kidneys are caused by this process (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. It is employed 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 possible therapeutic benefit in diabetic heart failure and kidney protection, it has drawn more interest in DN-related research (Qiao et al., 2017; Yonamine et al., 2016) found that by raising superoxide dismutase activity, RSV reduced oxidative stress in rats suffering from 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), 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 yet not is used in diabetes. So we have decided to use this combination for treatment of diabetes nephropathy in streptozocin induced diabetic rats. The present study was designed to evaluate the effect of co-administration of resveratrol with telmisartan on development and progression of diabetic nephropathy in experimentally-induced diabetes in experimental animals.

2. Materials and methods

2.1Experimental Animals:

Healthy male Wistar rats, weighing between 250 and 300 grams, were selected for the study. These animals were sourced from the K.B.H.S.S Institute of Pharmacy, located in Malegaon, Nashik. The animals were housed in well-ventilated polypropylene cages under controlled conditions, including a temperature of 25 ± 2°C and a 12-hour light/dark cycle. They were provided with regular pelletized feed (Nutrivet Life Science, Pune) and clean water. The study protocol was reviewed and approved by the institutional animal ethics committee, ensuring compliance with ethical standards. (IAEC No. KBH/2023/-02).

2.2 Drugs and Chemicals:

Streptozotocin (Ottochemie Laboratories, Mumbai, India), Telmisartan (Macleod's Laboratories, Bhilad, and Gujarat, India), Resveratrol (Sami Laboratories, Banglore), 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 divided into seven groups, with six animals in each group (n = 6). These groups underwent different drug treatments over a period of 45 days. The first group, labeled as the normal control group, received an oral dose of the vehicle (1% sodium carboxymethyl cellulose, Na-CMC) only. The second group, identified as the positive control group, was given 1% Na-CMC at a dose of 1 ml/kg orally.

once a day for 45 days. The 3rd and 4th group considered as standard group received telmisartan 5 mg/kg and 10 mg/kg PO respectively in 1 % Na-CMC once a day for 45 days. The 5th group of Animals were received resveratrol (5 mg/kg in 1% Na CMC in d.w., p.o.) daily from day 1 to day 45. While group of 6th and 7th animal were received telmisartan (5 mg/kg in 1% Na CMC in d.w., p.o.) + resveratrol (5 mg/kg in 1% Na CMC in d.w., p.o.) And telmisartan (10 mg/kg in 1% Na CMC in d.w., p.o.) + resveratrol (5 mg/kg in 1% Na CMC in d.w., p.o.) once a day 45 days respectively. Body weight was recorded on day 1st and day 45th of the treatment and on day 1st and day 45th of treatment, serum glucose (GOD/POD Method), serum triglyceride, serum total cholesterol, serum HDL- Cholesterol, HDL- cholesterol, serum albumin, serum creatinine, serum total protein, blood urea nitrogen, Hb1Ac were measured. Antioxidant enzymes assay was performed and measured at the end of experimental period.

After completion of treatment, the animals will be sacrificed by overdose of urethane and kidney samples will be dissected and rinsed in ice-cold saline. One kidney sample from each groups immediately fixed in a 10% formalin solution for histopathological analysis. Remaining Samples was used for estimation of oxidative parameters.

2.4 Preparation of STZ solutions

Streptozotocin (STZ) was dissolved in freshly prepared, ice chilled citrate buffer (pH 4.4). All the drug solution were freshly prepared before administration.

2.5 Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of streptozotocin, (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 level higher than 250 mg/dl was regarded as diabetic and involved in the study protocol.

2.6 Biochemical parameters from blood:

On day 1st and day 45th of treatment, all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in Eppendorf tubes. Serum was separated by centrifugation and was used for estimation of serum glucose (GOD/POD Method), serum triglyceride, serum total cholesterol, serum HDL- Cholesterol, HDL-cholesterol, serum albumin, serum creatinine, serum total protein, blood urea nitrogen, Hb1Ac using Meril diagnostic kit, India.

2.7 Study of morphometric parameters:

Body weight was recorded on day 1st and day 45th of the treatment. At end of study kidney was seperated and weighed.

2.8 Antioxidant enzymes assay

At the end of experimental period, remaining four the animals from each group were sacrificed with overdose of urethane. Kidney was removed and weighed. Kidney homogenates (5% w/v) was prepared in cold 30 mM Trisbuffer (pH 7.4) using Remi homogenizer so that clear homogenate is formed. The unbroken cells and cell debris was removed by centrifugation at 3000 rpm for 10 min using a Remi refrigerated centrifuge. The supernatant was

Used for the estimation of GSH, Lipid peroxidation (LPO), Na+/K+, NO, IL-1, IL-6, 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 added to 2.0 ml of freshly prepared 10% w/v Trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation and 2.0 ml of clear supernatant solution was mixed with 2.0 ml of freshly prepared Thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It

Was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532 nm against reagent blank. Different concentrations (0-23 nM) of standard malondialdehyde were taken and processed as above for standard graph. The values were expressed as nMol of MDA/mg tissue

2.9.2 Assay of Reduced Glutathione (GSH):

Equal volumes of tissue homogenate (supernatant) and 20% TCA was mixed. The precipitated fraction was centrifuged and to 0.25 ml of supernatant, 2ml of DTNB reagent was added. The final volume was made up to 3 ml with phosphate buffer. The colour developed was read at 412 nm against reagent blank. Different concentrations (10-50 µg) of standard glutathione were taken and processed as above for standard graph. The amount of reduced glutathione was expressed 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 incubates it for 15min at 37°C.Read the absorbance at 540nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

Standard curve:

Pipette into 100 ml volumetric flasks 0, 5, 10, 20 and 50 ml of nitrite standard (corresponding to 0, 2.5, 5, 10 and 25µg of nitrite) and dilute to about 80 ml with water. Add to each of the flasks 10ml of sulphanilamide solution and mix. After 3 min add 1 ml of coupling reagent, dilute to mark with water, mix and let stand for 15 min, Measure the absorbance of the solutions against water at 540 nm using 10 mm cuvettes. Draw a standard curve with the absorbance as a function of amount of nitrite (it shall be a straight line).

Sample:

Accurately weigh about 1g of the sample to the nearest 0.001g. Dissolve in water and dilute to 100 ml. Pipette 20 ml into a 100 ml volumetric flask and dilute to about 80 ml with water. Add 10 ml of sulphanilamide solution and mix. 3. After 3 min add 1 ml of coupling reagent, dilute to mark with water, mix and let stand for 15 min. 4. Measure the absorbance of the solution against water at 540 nm using 10 mm cuvettes. 5. Read on the standard curve the amount of nitrite corresponding to the actual absorbance.

2.10 Pro-inflammatory cytokinins:

The serum was sent to Shree bios innovation laboratory, Pune, India for cytokinine level detection and were done according to the manufacturers protocol.

2.11 Histopathological examination:

The Kidney tissue was removed from the Rat immediately when they were sacrificed in order to conduct histological analysis. 1 kidney specimens were fixed in 10% phosphate-buffered formalin, dehydrated in graded alcohol, and embedded in paraffin sections. And those Kidneys samples were sent to Shree bios innovation laboratory, Pune, India.

2.12 Statistical analysis:

One-way analysis of variance (ANOVA) was be applied for statistical analysis with post hoc Bonferroni's multiple comparison for hemodynamic parameters and Data for each parameter was be analysed by one way ANOVA followed by Dunnett's post hoc test using a graph pad, 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 showed a significant increase in serum glucose levels compared to the normal control group (###p<0.001), demonstrating severe hyperglycaemia typical of diabetes. Treatment of telmisartan at dose 5 (***p < 0.001) & 10(***p < 0.001) mg/kg showed significant reduction in blood glucose level time on 45th day when compared to disease control group, The higher dose of telmisartan proved slightly more effective than the lower dose in reducing hyperglycaemia Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in blood glucose level time on 45th day when compared to disease control group, (fig-1). Telmisartan + Resveratrol (TR1): Combined treatment with TEL-1 and resveratrol significantly improved serum cholesterol levels compared to the diabetic control group (***p<0.001), this group glucose levels were almost restored to normal control levels, indicating a highly effective synergistic effect. Telmisartan + Resveratrol (TR2): Combined treatment with TEL-2 and resveratrol resulted in the most significant reduction in serum glucose levels, almost reaching normal control levels. The reduction was significant compared to the diabetic control group (***p < 0.001).

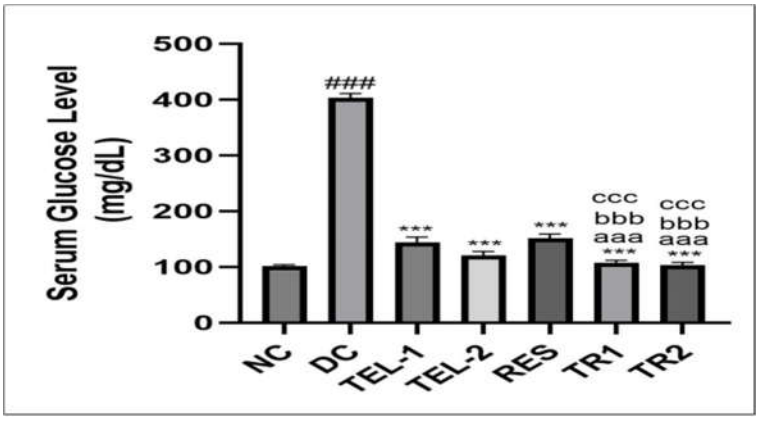


Fig 1: Effect of telmisartan and resveratrol on serum glucose in STZ-induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. ###p<0.001when compared to normal control (NC); ***p<0.001 when compared to diabetic control (DC); aaap<0.001 when compared to TEL1 group; bbbp<0.001 when compared to TEL2 group and cccp<0.001 when compared to RES group.

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

As depicted in fig-2, the serum cholesterol level in disease control group was significantly increased at all when compared to normal control group (###p<0.001). The low dose of telmisartan (5 mg/kg) showed significant reduction in serum cholesterol level than high dose of telmisartan on 45th day when compared to disease control group (***p < 0.001) &(***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in serum cholresterol level time on 45th day when compared to disease control group but less significant than telmisartan 5 mg/kg dose.

Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced serum cholesterol levels compared to the DC group (***p<0.001). Moreover, this combination showed highly significant reductions compared to TEL-2 (bbbp<0.001), and Resveratrol (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) also resulted in a significant reduction in serum cholesterol levels compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL-2 (aaap<0.001), Resveratrol (cccp<0.001) groups.

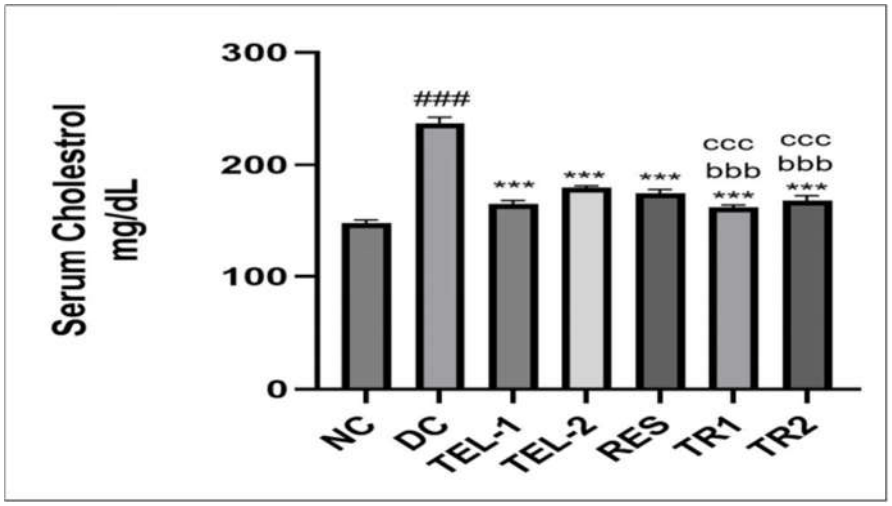


Fig 2: Effect of telmisartan and resveratrol on serum cholesterol in STZ-induced diabetes in rats.

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol. Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. ###p<0.001when 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 depicted in fig-3, the serum triglyceride level in disease control group was significantly increased at all when compared to normal control group (###p<0.001). The low dose of telmisartan (5 mg/kg) showed significant reduction in serum triglyceride level than high dose of telmisartan (10 mg/kg) on 45th day when compared to disease control group (***p < 0.001) & (***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in serum triglyceride level on 45th day when compared to disease control group. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced serum triglyceride levels compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.001) and RES (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant reduction in serum triglyceride levels compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and significantly superior to the TEL2 group (bbbp<0.001). The last group Telmisartan + Resveratrol (TR2) showing more prominent effet than other groups.

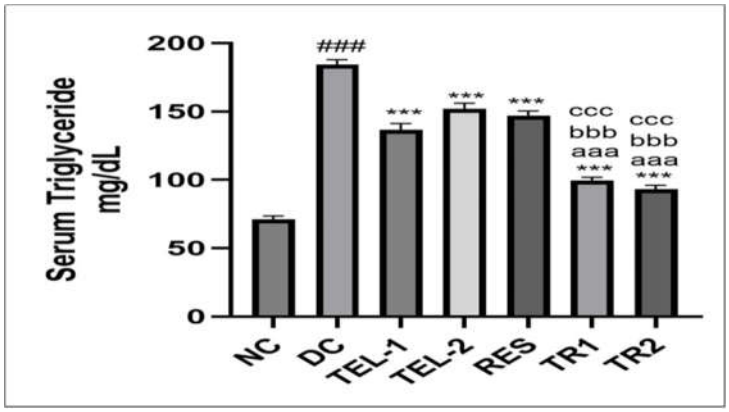


Fig 3: Effect of telmisartan and resveratrol on serum triglyceride in STZ-induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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 diabetic control group showed significantly reduced HDL-cholesterol levels. This substantial decrease (###p<0.001 compared to NC) highlights the adverse impact of diabetes on lipid metabolism, particularly in lowering protective HDL-cholesterol levels. Treatment with Telmisartan 5 mg/kg (***P < 0.001) & 10 (***P < 0.001) mg/kg increased HDL-cholesterol significantly when compare to disease control group)(fig-4). But when compare to telmisartan lower dose to high dose, lower dose shows more proven action than high dose. The resveratrol-treated group displayed HDL- cholesterol levels which was significantly higher than those in the DC group (***p<0.001 compared to DC). This demonstrates the effectiveness of resveratrol in improving HDL- cholesterol levels, approaching those of the Normal control group. This treatment resulted in HDL- cholesterol levels significantly higher than the DC group (***p<0.001 compared to DC) and also significantly higher than the TEL-2 group (***p<0.001 compared to TEL-2). Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly increased HDL-cholesterol levels compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL2 group (bbbp<0.001) and RES (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant improvement in HDL-cholesterol levels compared to the DC group (***p<0.001). This reduction was also highly significant compared to the RES (cp<0.05) group and significantly superior to the TEL2 group (bbbp<0.001). The Telmisartan + Resveratrol (TR1) showing more prominent effet than other groups, seems greater potential in improving lipid profiles.

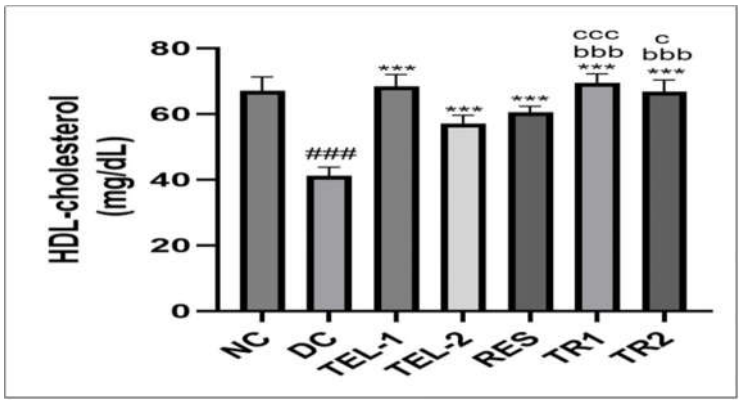


Fig 4: Effect of telmisartan and resveratrol on HDL-cholesterol in STZ induced diabetes in rats

NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol. Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when compared to Normal control (NC); ***p<0.001 when compared to diabetic control (DC); ***p<0.001 compared to disease control group; bbbp<0.001 when compared to TEL2 group and cp<0.05 when compared to RES group.

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

The diabetic control group showed a significant increase in LDL-cholesterol levels compared to the normal control group (###p<0.001). Treatment of telmisartan at dose 5 (***p < 0.001) & 10(***p < 0.001) mg/kg showed significant reduction in LDL-cholesterol level time on 45th day when compared to disease control group, The both dose of telmisartan proved slightly more effective than the Resveratrol dose. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in LDL-cholesterol level time on 45th day when compared to disease control group. Both combination groups (TR1 and TR2) showed significant reductions in LDL-cholesterol levels compared to the DC group, with p-values < 0.001 (***p < 0.001). Although both combination groups seems more significant effect than single dose of telmisartan and Resveratrol. The most pronounced reduction in LDL-cholesterol levels was observed in the last group, highlighting the enhanced benefit of combining higher doses of this compound (fig-5).

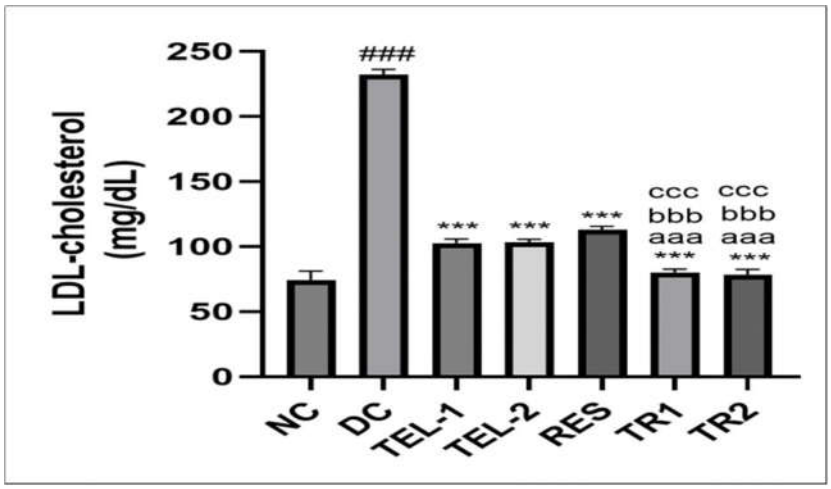


Fig-5 Effect of telmisartan and resveratrol on LDL-cholesterol in STZ induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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. 6 Effect of telmisartan and resveratrol on serum creatinine in STZ induced diabetes in rats.

As depicted in fig-6, the serum creatinine level in disease control group was significantly increased at all when compared to normal control group (###p<0.001). The high dose of telmisartan (10 mg/kg) showed significant reduction in serum Creatinine level than lower dose of telmisartan (5 mg/kg) mice on 45th day when compared to disease control group (***p < 0.001) & (***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in serum creatinine level time on 45th day when compared to disease control group. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced serum creatinine levels compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.05) and RES (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant reduction in serum creatinine levels compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and significantly superior to the TEL2 group (bbbp<0.001).

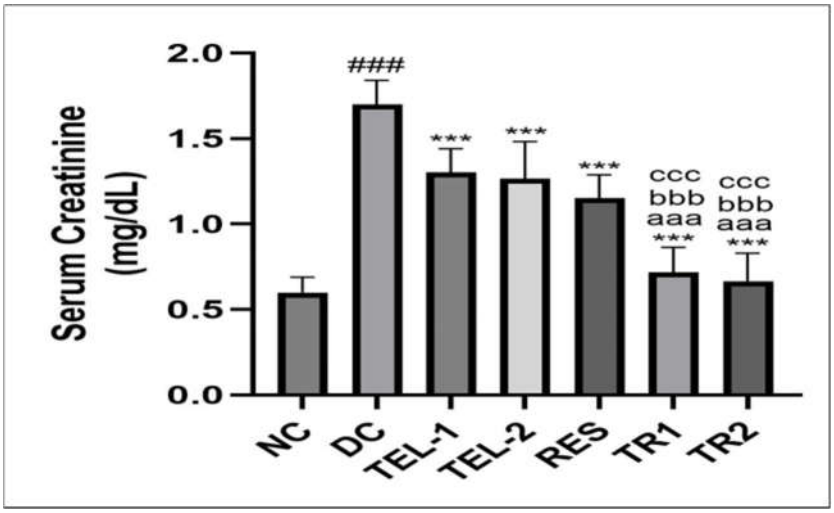


Fig-6 Effect of telmisartan and resveratrol on serum Creatinine in STZ induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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 significantly higher BUN levels indicate severe kidney dysfunction as a result of diabetes, The diabetic control group showed a significant increase in serum BUN levels compared to the normal control group (###p<0.001), demonstrating severe kidney dysfunction typical of diabetes. Treatment of telmisartan at dose 5 (***p < 0.001) & 10(***p < 0.001) mg/kg showed significant reduction in blood BUN level time on 45th day when compared to disease control group, The higher dose of telmisartan proved slightly more effective than the lower dose. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in blood serum BUN time on 45th day when compared to disease control group, (fig-7) Telmisartan + Resveratrol (TR1): Combined treatment with TEL-1 and resveratrol significantly improved serum BUN level compared to the diabetic control group (***p<0.001), this groups serum BUN levels were look likes to normal control levels, indicating a highly effective synergistic effect. Telmisartan + Resveratrol (TR2): Combined treatment with TEL-2 and resveratrol resulted in the most significant reduction in serum BUN level, almost reaching normal control levels. The reduction was significant compared to the diabetic control group (***p < 0.001).

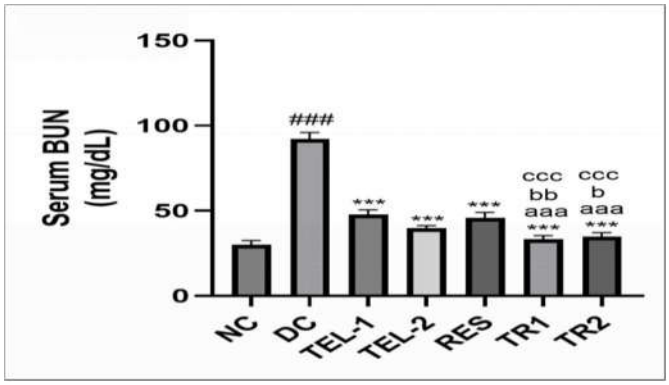


Fig-7 Effect of telmisartan and resveratrol on serum BUN in STZ induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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 diabetic control group showed significantly reduced serum albumin levels (###p<0.001 compared to NC). Animals received a lower dose of telmisartan significantly increases serum albumin levels indicating a significant improvement compared to the DC group (***p<0.001 compared to DC). The higher dose of telmisartan resulted in serum albumin levels showing an even greater improvement and a highly significant increase compared to the DC group (***p<0.001 compared to DC) also highly effective than lower dose of telmisartan. These results suggest a dose-dependent effect of telmisartan in enhancing serum albumin levels in diabetic rats. The resveratrol-treated group displayed serum albumin levels which was significantly higher than those in the DC group (***p<0.001 compared to DC). This demonstrates the effectiveness of Resveratrol in improving serum albumin levels,

approaching those of the normal control group (***p<0.001). Animal received Low dose combination of telmisartan and resveratrol resulted in serum albumin levels significantly higher than the DC group (***p<0.001 compared to DC), also significantly higher than the TEL-1 group (aaap<0.01 compared to TEL-1) and significantly higher than the resveratrol group (cccp<0.01 compared to resveratrol). Rats receiving the second novel treatment had serum albumin levels increased highly significant compared to the DC group (***p<0.001 compared to DC) and showed further significant improvements over both TEL-1 and TEL-2 groups (aaap<0.001 compared to TEL-1, (bp<0.01 compared to TEL-2) respectively and significantly higher than the resveratrol group (cccp<0.01 compared to resveratrol), (Fig-8).

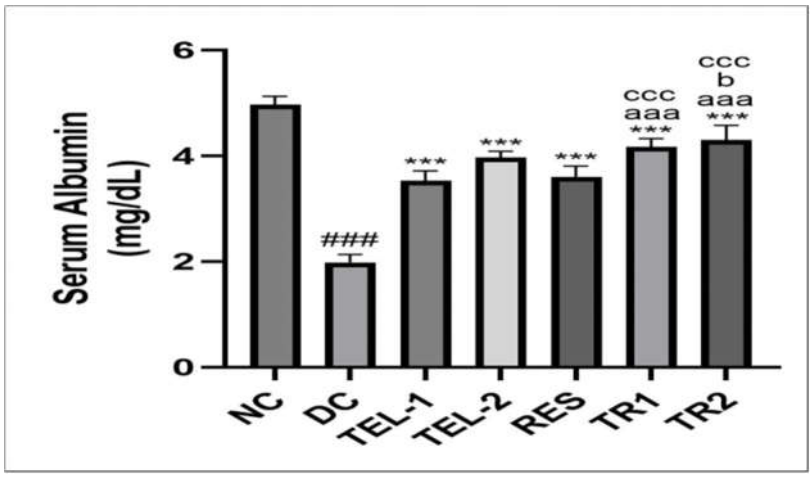


Fig-8 Effect of telmisartan and resveratrol on serum Albumin in STZ induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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 STD induced diabetes in rats.

The diabetic control group showed a significant increase in serum HbA1c level compared to the normal control group (###p<0.001). Treatment of telmisartan at dose 5 (***p < 0.001) & 10(***p < 0.001) mg/kg showed significant reduction in serum HbA1c l time on 45th day when compared to disease control group, The both dose of telmisartan proved slightly more effective than the Resveratrol dose. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in serum HbA1c level time on 45th day when compared to disease control group. Both combination groups (TR1 and TR2) showed significant reductions in serum HbA1c levels compared to the DC group (***p < 0.001). Although both combination groups seem more significant effect than single dose of telmisartan and Resveratrol. The most pronounced reduction in serum HbA1c levels was observed in the TR2 group, which showing more significant action than TEL-1, TEL-2 and RES groups(fig-9).

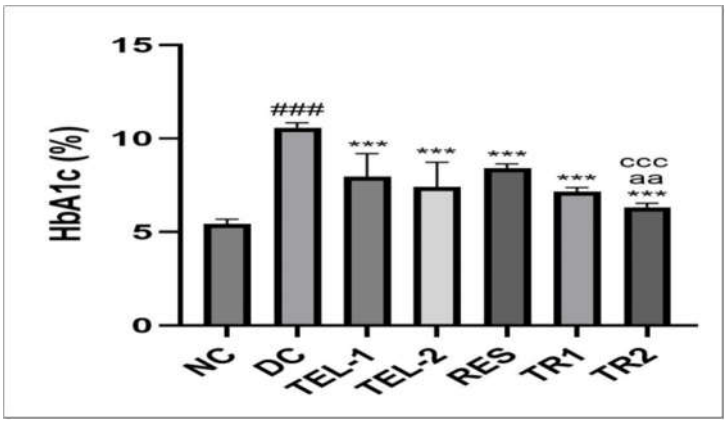


Fig-9 Effect of telmisartan and resveratrol on serum HbA1c in STZ induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. ###p<0.001when 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 depicted in fig-10, the CRP level in disease control group was significantly increased at all when compared to normal control group (###p<0.001). The high dose of telmisartan (10 mg/kg) showed significant reduction in CRP level than lower dose of telmisartan (5 mg/kg) mice on 45th day when compared to disease control group (***p < 0.001) & (***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in CRP level time on 45th day when compared to disease control group. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced CRP level compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.05) and RES (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant reduction in CRP level compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and significantly superior to the TEL2 group (bbbp<0.001).

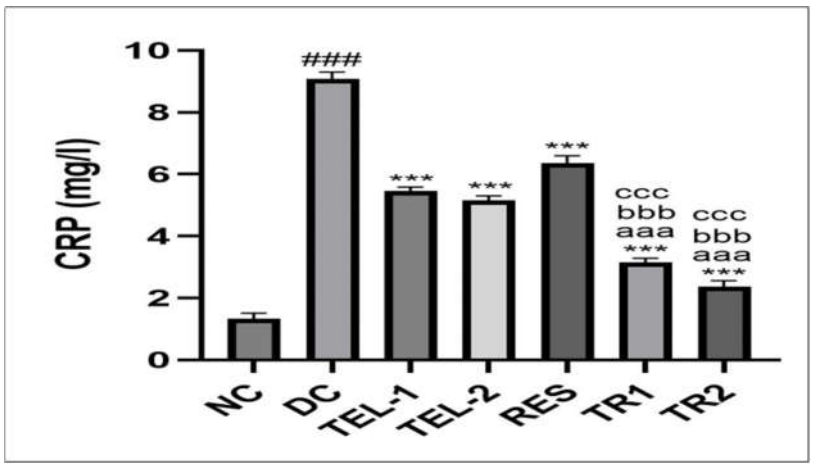


Fig-10 Effect of telmisartan and resveratrol on CRP in STZ induced diabetes in rats:

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. ###p<0.001when 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.

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

DC group shows a significant increase in TNF-α levels compared to the normal control group, indicating heightened systemic inflammation associated with diabetes (###p<0.001when compared to normal control). Treatments with telmisartan at a dose of 5 mg/kg results in a significant reduction in TNF-α levels compared to the diabetic control group (DC). A higher dose of telmisartan (10 mg/kg) results in a more substantial reduction in TNF-α level compared to both the DC and TEL-1 groups. Animal were treated with resveratrol at 5 mg/kg results in a reduction in TNF-α levels compared to the DC group. This reduction is statistically significant (***p<0.001), indicating that resveratrol helps to reduce inflammation, although it is less effective compared to the higher dose of telmisartan. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced TNF-α levels compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL1 (aaap<0.001), TEL2 group (bbbp<0.05) and RES (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant reduction in TNF-α level compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and significantly superior to the TEL2 group (bbbp<0.001) (fig-11).

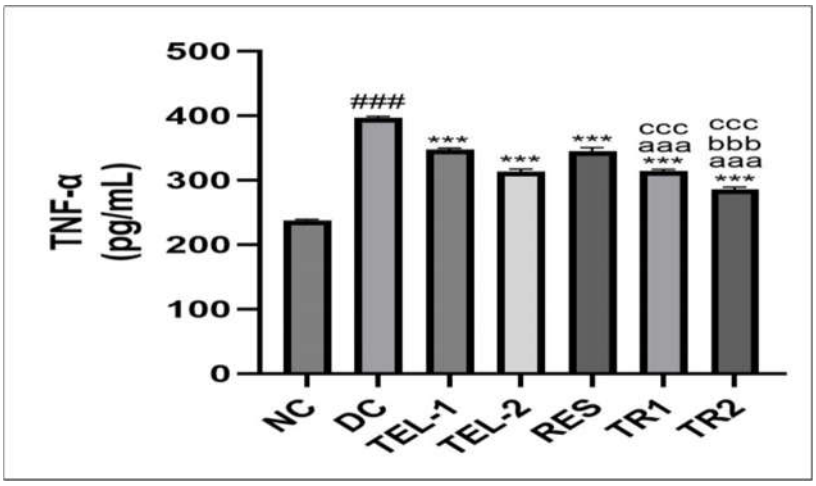


Fig-11 Effect of telmisartan and resveratrol on TNF-α in STZ induced diabetes in rats:

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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.

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

DC group shows a significant increase in IL-1β levels compared to the normal control group, indicating heightened systemic inflammation associated with diabetes. The "###" indicates that this increase is highly significant (###p<0.001) compared to the normal control group (NC). Treatment of telmisartan at dose 5 (***p < 0.001) & 10(***p < 0.001) mg/kg showed significant reduction in IL-1 β level time on 45th day when compared to disease control group, telmisartan 10 mg/kg proved slightly more effective than the telmisartan low dose andResveratrol dose. Treatment of Resveratrol at dose 5 mg/kg showed significant reduction in IL-1 β level (***p < 0.001) time on 45th day when compared to disease control group. Both combination groups (TR1 and TR2) showed significant reductions in IL-1 β levels compared to the DC group, with p-values < 0.001 (***p < 0.001). Although both combination groups seems more significant effect than single dose of telmisartan and Resveratrol. The most pronounced reduction in IL-1 β levels was observed in the TR2 group, highlighting the enhanced benefit of combining higher doses of this compound (fig-12).

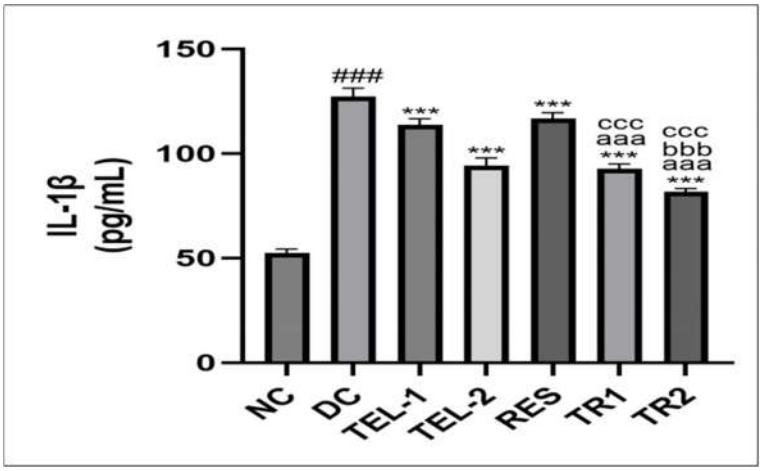


Fig-12 Effect of telmisartan and resveratrol on IL-1 β in STZ induced diabetes in rats:

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. ###p<0.001when 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

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

As depicted in fig-13, the IL-6 level in disease control group was significantly increased at all when compared to normal control group (###p<0.001). The high dose of telmisartan (10 mg/kg) showed significant reduction in IL-6 level than lower dose of telmisartan (5 mg/kg) mice on 45th day when compared to disease control group (***p < 0.001) & (***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in IL-6 level time on 45th day when compared to disease control group and seems similar effective as telmisartan 5 mg/kg. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced IL-6 level compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL1 (aaap<0.001), TEL2 group (cccp<0.001 and RES (cccp<0.001) groups. Telmisartan + Resveratrol (TR2): The combination of a

higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant reduction in IL-6 level compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and significantly superior to the TEL2 group (bbbp<0.001).

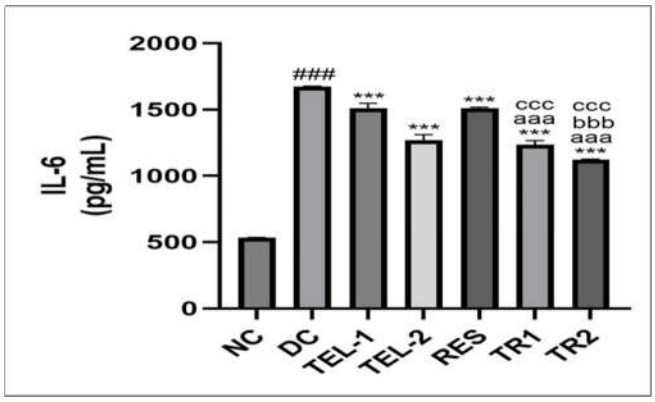


Fig-13 Effect of telmisartan and resveratrol on IL-6 in STZ induced diabetes in rats:

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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.

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

As depicted in fig-14, the body weights in disease control group was significantly decrease at all when compared to normal control group (###p<0.001). Treatment with telmisartan (5 mg/kg) and (10 mg/kg) showed significant increase in body weights on 45th day when compared to disease control group (***p < 0.001) & (***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant increased in body weights on 45th day when compared to disease control group. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significant increased in body weights compared to the DC group (***p<0.001). Additionally, this combination showed significant increases 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) resulted in a significant increased in body weights compared to the DC group (***p<0.001). This increases weight was also highly significant compared to the TEL1 (aaap<0.001) and RES (cccp<0.001) groups, and significantly superior to the TEL2 group (bp<0.05).

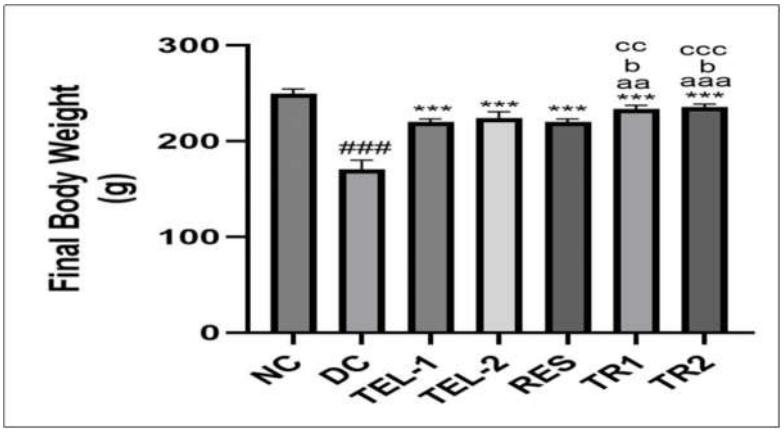


Fig-14: Effect of telmisartan and resveratrol on body weight (g) in STZ induced diabetes in rats.

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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 Effect of telmisartan and resveratrol on kidney weight (g) and kidney hypertrophy index in STZ induced diabetes in rats.

As depicted in fig-15, the kidney weight in disease control group was significantly increased at all when compared to normal control group (###p<0.001). The high dose of telmisartan (10 mg/kg) showed significant reduction in kidney weight than lower dose of telmisartan (5 mg/kg) mice on 45th day when compared to disease control group (***p < 0.001) & (***p < 0.001) respectively. Treatment of Resveratrol at dose 5 (***p < 0.001) mg/kg showed significant reduction in kidney weight on 45th day when compared to disease control group and seems more effective as telmisartan 5 mg/kg. Telmisartan + Resveratrol (TR1): The combination of telmisartan (5 mg/kg) and resveratrol (5 mg/kg) significantly reduced kidney weight compared to the DC group (***p<0.001). Additionally, this combination showed significant reductions compared to the TEL2 group (bbp<0.01) groups. Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) resulted in a significant reduction in kidney weight compared to the DC group (***p<0.001). This reduction was also highly significant compared to the TEL1 (aap<0.01).

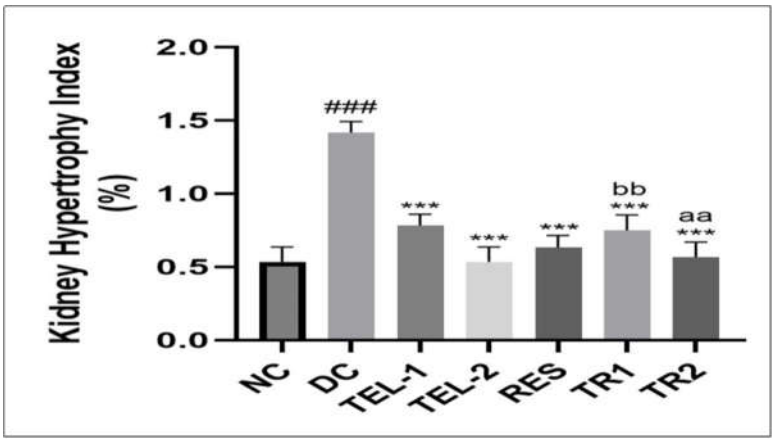


Fig-15 Effect of telmisartan and resveratrol on kidney weight (g) and kidney hypertrophy index in STZ induced diabetes in rats

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

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparisons. ###p<0.001when 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

As shown in table 1 STZ induced diabetes group significantly increases LPO and serum K+ level compared to (###P < 0.001) and significantly decrease GSH (###P < 0.001), NO (###P < 0.001) and serum Na+ as compared to normal control, treatment of Telmisartan 5 mg/kg and 10 mg/kg for 45 days

significantly decrease the LPO and serum K+ level (***P < 0.001 and ***P < 0.001 respectively) and significantly increased GSH Levels (***P < 0.001 and ***P < 0.001 respectively) NO levels (***P < 0.001 and ***P < 0.001 respectively) as compared to disease control group. While group received resveratrol 5 mg/kg for 45 days significantly decrease the LPO and serum K+ Levels (***P < 0.001) and increased GSH Level but not significant as telmisartan group (*P < 0.05) but significantly increase NO levels (***P < 0.001) as compared to disease control group, although Telmisartan (5 mg/kg) + Resveratrol (5 mg/kg) (TR1): This combined therapy shows a highly significant improvement (***p<0.001) over the DC group.Telmisartan + Resveratrol (TR2): The combination of a higher dose of telmisartan (10 mg/kg) with resveratrol (5 mg/kg) yields the most significant reduction in LPO levels, lower than any other group and increase the 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

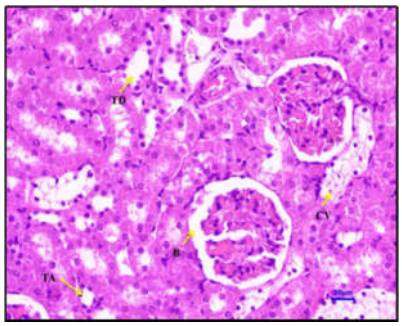
NC- Normal control, DC- Disease control, TEL-1- Telmisartan, TEL-2- Telmisartan, RES- Resveratrol, TR1- Telmisartan + Resveratrol, TR2- Telmisartan + Resveratrol

Data are presented as means ± SD (n=6/group). One-Way ANNOVA followed by Bonferroni test for multiple comparison. ###p<0.001when compared to normal control (NC); ***p<0.001, *p<0.05 when compared to diabetic control (DC); aaap<0.001), aap<0.01 & ap<0.05 compared to TEL1 group; bbbp<0.001 & bbp<0.01 when compared to TEL2 group and cccp<0.001 when compared to RES group.

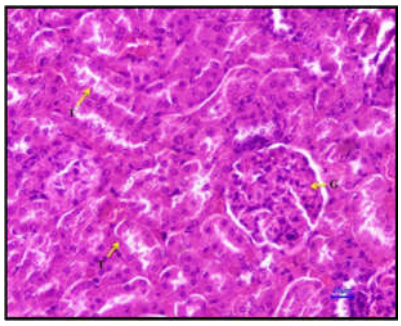
3.17 Effect of telmisartan and resveratrol on Histopathology of Kidney in STZ induced diabetes in rats

Effect of telmisartan and resveratrol on Histopathology of Kidney in STZ induced diabetes in rats is shown in fig-16 the histopathological examination reveals that Micrograph for Kidney showing changes in Tubular Dilation (TD), Tubular atrophy (TA), Dilated bowman's space (B), Cytoplasmic vacuolation (CV)

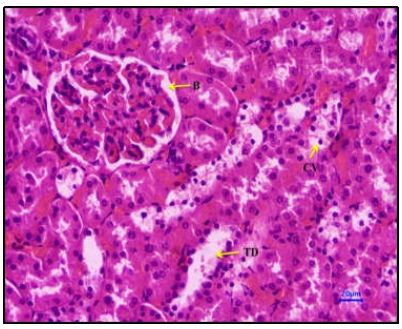
As a result, from the fig-16 Kidney histopath, pathological changes in kidney due to the treatment of STZ induced DN, which is significantly Attenuated by the treatment of telmisartan and resveratrol



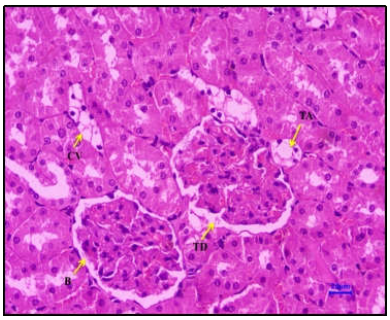
1 Normal control



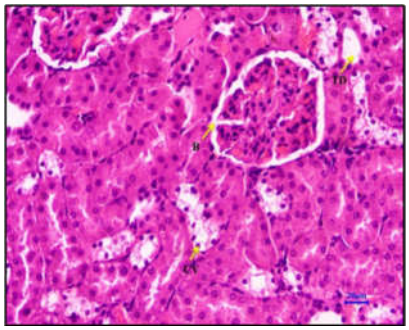
2 Diabetic control



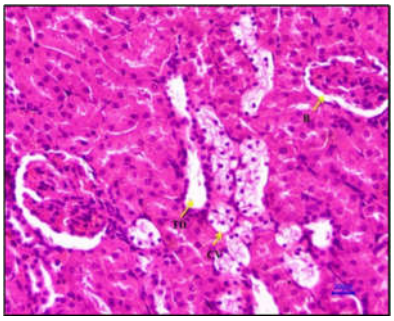
3. Telmisartan- I (5 mg/kg)



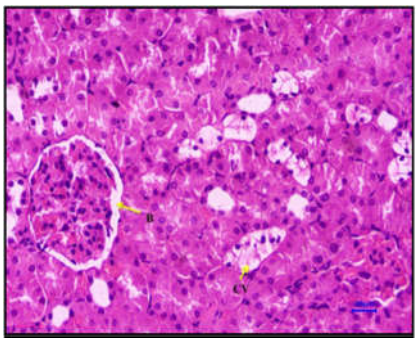
4. Telmisartan- I (10 mg/kg)



5. Resveratrol (5 mg/kg)



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



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

Kidney tissue showed Normal histology, Renal Tubule (T) and Glomeruli (G), Tubular Dilation (TD), Tubular atrophy (TA), Dilated bowman's space (B), Cytoplasmic vacuolation (CV)

Discussion:

With over 100 million cases globally (6% of the global population), diabetes mellitus (DM) is the most common endocrine circumstances. It causes in 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 is hazardous to the beta cells of the pancreas, which are in charge of making 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 diabetics through destruction of pancreatic beta cells, Via glucose transporter 2 (GLUT2), the STZ reaches pancreatic beta cells and damages DNA by producing 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 plays a crucial role in the development and progression of diabetic nephropathy (DN). Persistent hyperglycemia leads to oxidative stress, which is a key factor in the pathophysiology of DN and kidney damage (Liang et al., 2021). Studies have shown that controlling blood glucose levels through insulin treatment can mitigate renal fibrosis by stabilizing the expression of protein, a negative regulator of the TGF-β1/Smads signalling 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. Moreover, it has been discovered that controlling carbohydrates and energy intake can mitigate kidney damage by decreasing oxidative stress and averting hypoxia by increasing HIF-1α levels (Mahajan et al., 2020). These findings highlight the significance of glucose control in preventing and managing DN.

Telmisartan, an angiotensin II receptor blocker, demonstrates protective effects in diabetic nephropathy by targeting multiple pathways. It ameliorates high-glucose-induced injury in renal glomerular endothelial cells by antagonizing angiotensin II type 1 receptor (AT1R) (Zhan et al., 2021), reduces oxidative stress, inflammation, and apoptosis through Nrf2/HO-1 signalling (Antar et al., 2022), Telmisartan also improves kidney function by affecting mitochondrial oxidative phosphorylation, the peroxisome proliferator-activated receptor (PPAR) signalling pathway, and the slit diaphragm (Wu et al., 2013).

Resveratrol is a natural polyphenol found in red grapes and other plant sources that has been shown to have beneficial effects on cholesterol levels and diabetic nephropathy. resveratrol works to improve

cholesterol levels in diabetic nephropathy is through its ability to activate the SIRT1 pathway (Palsamy et al., 2010). SIRT1 is a protein that plays a key role in regulating lipid metabolism and cholesterol levels in the body (Gao et al., 2018). Resveratrol can increase the activity of SIRT1, which in turn helps to lower cholesterol levels by reducing the synthesis of cholesterol in the liver and increasing the breakdown of cholesterol in the blood (Jung et al., 2014).

Resveratrol improves renal function by reducing blood glucose levels, inflammation, and oxidative stress (Cai et al., 2020). Additionally, resveratrol exerts anti-fibrotic, anti-inflammatory, and anti-oxidative stress properties, leading to improvements in renal indicators such as blood urea nitrogen, serum creatinine, and proteinuria (Xian et al., 2020 It is observed that serum level of total cholesterol, LDL, and triglyceride was significantly decreased after animals treated with resveratrol. Hence by using this mechansim the combination telmisartan and resveratrol may reduce the blood glucose level.

Total protein level is an important 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, serum creatinine, serum albumin, and blood urea nitrogen (BUN)which is a marker 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).in present study demonstrated that Animal treated with STZ significantly increase proteins levels and ameliorating by using different treatment of telmisartan 5 and 10 mg/kg, however Resveratrol treatment significantly decreased total protein levels in the urine of diabetic rat (Chen et al., 2015). Resveratrol exerted its protective effects by reducing the expression of pro-inflammatory cytokines and oxidative stress markers, thereby preventing damage to the renal tissue and leakage of proteins into the urine. Although by using its combination of telmisartan and resveratrol show highly significant reduction of protein levels.

Creatinine is a waste product produced by muscles from the breakdown of creatine phosphate, and it is typically filtered out of the blood by the kidneys and excreted in the urine. In the context of diabetic nephropathy, which is a progressive kidney disease that occurs in people with diabetes, creatinine levels can be indicative 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 increased serum creatinine concentration is associated with induction oxidative stress and blocking the AKT signalling pathway, which is reduced by ameliorating oxidative stress by giving treatment of telmisartan and resveratrol, although combination of this both showing more effective than single doses.

Blood Urea Nitrogen (BUN) is a commonly used marker to assess kidney function, as it reflects the levels of urea in the blood that are normally 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 have been associated with a higher risk of progression of diabetic nephropathy and can be used as a prognostic marker for the development of chronic kidney disease in diabetes (Kim et al., 2021).in present study BUN is increased in disease control group is associated with the oxidative stress and glomerular filtration impairment. 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 drug's effects on rat diabetic nephropathy, the antioxidant characteristics of resveratrol, which can help to protect the kidneys from harm caused by oxidative stress, (Kim et al., 2011).

A strong association between elevated HbA1c levels and the development and progression of diabetic nephropathy in both animal models and human patients (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 diabetic control group increased HbA1c levels and which is significantly mitigated by Telmisartan and resveratrol doses. telmisartan may reduce HbA1c levels in diabetic nephropathy in rats through its ability to block the AT1 receptor, reduce inflammation and oxidative stress, improve insulin sensitivity, and lipid profiles (Vanholder et al., 2018). Resveratrol in reducing HbA1c levels in diabetic nephropathy involves its ability to modulate glucose metabolism and ability of resveratrol to enhance insulin sensitivity and suppress inflammation in the kidney (Wu et al., 2017).

The manifestation in kidney injury has been observed to be correlated with CRP. In the kidneys of diabetic rats, elevated CRP levels have been linked to increased oxidative stress, inflammation, and tissue damage. Furthermore, studies have demonstrated that in rat models, lowering CRP levels might enhance renal function and lessen the severity of diabetic nephropath (Navarro-González et al., 2011). In the current study shown that increase CRP level in STZ induced diabetic rat which is ameliorating by continuous administration Telmisartan, resveratrol and combination of both for 45 days. Telmisartan exerts its protective effects by continuous administration in diabetic nephropathy through its ability to block the AT1 receptor, reduce inflammation and fibrosis, and improve renal function (Xu et al., 2011). Resveratrol is a known activator of SIRT1, a NAD+-dependent histone deacetylase that regulates various cellular processes, including inflammation. SIRT1 activation by resveratrol can suppress CRP expression by inhibiting the acetylation of NF-kB (Sharma et al., 2022).

It is also well establish that consecutive use of STZ leads to increase oxidative stress Kidney and other part of body , the present study show that administration STZ significantly increased oxidative stress in the Kidney of Rat, as evidenced by decreased activity of antioxidant enzymes such as GSH,NO and Sodium and increase in LPO and potassium levels .Oxidative stress has been recognized as a key player in the pathogenesis of diabetic nephropathy (DN), a complication of diabetes characterized by kidney damage. In present study Telmisartan and resveratrol has been shown to have antioxidant effects by reducing oxidative stress and inflammation in various 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). although the combination of this both drugs having more effect, than single dose.

In the present study NO level significantly decreased in STZ induced diabetic rats which are ameliorated by telmisartan and Resveratrol by continuous dosing for 45 days. NO plays a significant 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), the enzyme responsible for producing NO in the endothelium.

One important anti-inflammatory cytokine in the context of diabetic nephropathy is interleukin-10 (IL-10). IL-10 is known to have potent anti-inflammatory effects and can inhibit the production of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in the kidneys. Studies have shown that administration of IL-10 can attenuate renal inflammation and fibrosis in diabetic nephropathy models in rats (Tesch et al., 2008). By blocking the angiotensin II type 1 receptor, telmisartan prevents the activation of downstream pathways that lead to the production and release of pro-inflammatory cytokines. Additionally, telmisartan has been shown to upregulate the expression of anti-inflammatory cytokines, such as interleukin-10 (IL-10), which helps to counteract the inflammatory response (Rodriguez-Naranjo et al., 2013). Resveratrol exerts its anti-inflammatory effects by modulating various signalling pathways and reducing the expression of pro-inflammatory cytokines, while increasing the expression of anti-inflammatory cytokines (Xin et al., 2024).

Resveratrol was shown to reduce the levels of pro-inflammatory cytokines such as TNF-α and IL-6 in a rat model of diabetic nephropathy (Gulcubuk et al., 2014). Hence the combination of telmisartan and resveratrol can reduce the pro-inflammatory cytokines in diabetic rats.

Examination of kidney tissues from the normal control group of rats showed no significant lesions. In contrast, rats in the disease control group displayed mild to moderate nephropathic lesions, including cytoplasmic vacuolation, tubular dilation, tubular atrophy, and Bowman's space dilation. Treatment with telmisartan and resveratrol combination (T1R) or lower doses of the drug (T2R) reduced the severity and extent of these lesions, with T1R showing greater effectiveness. This suggests that the drug treatment has a beneficial impact on kidney health in these rats.

Diabetic nephropathy (DN) remains one of the most serious microvascular complications associated with diabetes mellitus, primarily due to persistent hyperglycemia-induced oxidative stress and inflammation. In the present study, we assessed the protective role of Telmisartan and resveratrol, individually and in combination, in a streptozotocin (STZ)-induced diabetic rat model. The rationale for selecting these agents lies in their established pharmacological profiles: Telmisartan, an angiotensin II receptor blocker (ARB), is known for its renoprotective and anti-inflammatory properties, while resveratrol, a natural polyphenolic compound, exhibits strong antioxidant and anti-diabetic effects.

The biochemical results of our study demonstrated that both Telmisartan and resveratrol, when administered individually, significantly ameliorated hyperglycemia and restored altered serum markers related to kidney function, such as 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.

Oxidative stress plays a central role in the pathogenesis of diabetic nephropathy. Our findings revealed that combination treatment markedly reduced malondialdehyde (MDA) levels and significantly increased the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), compared to individual treatments. This suggests that the enhanced antioxidant capacity of the combination therapy could be a major contributing factor to its nephroprotective effects.

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. Treatment with Telmisartan and resveratrol, especially in combination, resulted in significant improvement in renal architecture, indicating effective attenuation of renal damage.

The combination of Telmisartan and resveratrol at 10 + 5 mg/kg emerged as the most effective treatment. The superior efficacy of this dose can be attributed to the dual mechanism of action—Telmisartan’s inhibition of the renin-angiotensin system (RAS) and resveratrol’s antioxidant and anti-inflammatory properties. Notably, Telmisartan is also a partial agonist of PPAR-γ, which may further enhance insulin sensitivity and exert additional protective effects on 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 avenues for potential combination therapies that leverage both pharmaceutical and natural agents for managing diabetic complications more effectively.

Conclusion

In the present work, we examine the preventive effect of Telmisartan and resveratrol against STZ induced diabetes in rats in a dose-dependent and combined dose manner (5 and 10 mg/kg) and 5 mg/kg

respectively and combination of Telmisartan and resveratrol (5 + 5 mg/kg and 10 + 5 mg/kg) respectively. Based on Biochemical, oxidative, and Histopathological examination, we conclude that the most appropriate combination dose of is Telmisartan and resveratrol (10 + 5 mg/kg), showing strong combination treatment for Diabetic nephropathy

Abbreviations	full forms of Abbreviations
NC	Normal control
DC	Disease control
TEL-1	Telmisartan 5 mg/kg
TEL-2	Telmisartan 10 mg/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

Funding:

None

Conflicts of interest:

None

Ethics approval:

The experimental protocol was approved by 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, checking, editing and all revisions of the manuscript. Smruti R. Jadhav contributed in carrying out all the experiments, statistical analysis of the data and preparing the first draft of the manuscript including table and figures. Shekhar D. Jagtap, Swapnil B. Jadhav and Vinod A. Bairagi contributed in preparing and implementation of study design and also contributed in data analysis.

References:

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.

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.

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.

Samsu, N., 2021. Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. BioMed research international, 2021(1), p.1497449.

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.

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.

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.

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.

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.

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.

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.

King, G.L., 2008. The role of inflammatory cytokines in diabetes and its complications. Journal of periodontology, 79, pp.1527-1534.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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).