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Retatrutide Treatment in Streptozotocin-Induced Diabetic Rats: Renal Inflammatory Cytokines, HIF-1 α Signalling, and Histopathological Injury Profile

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KEYWORDS:

Diabetic Nephropathy, STZ-induced Diabetes, HIF-1 α , Early Renal Injury, Inflammation

MAIN POINTS:

- Model validity and inflammatory signature:** Streptozotocin-induced diabetes produced a robust early diabetic kidney injury model, evidenced by significantly increased IL-1 levels and marked cortical and medullary histopathological damage in the diabetic group compared with controls.
- Limited but non-significant structural benefit of Retatrutide:** RET did not provide statistically significant histopathological protection, but STZ+RET animals consistently showed numerically lower cortical and medullary scores than S.
- Retatrutide modulates hypoxia-related molecular responses:** HIF-1 gene expression was significantly elevated in diabetic kidneys and significantly reduced in both Retatrutide-treated groups compared with the diabetic group, suggesting an early modulatory effect of Retatrutide on hypoxia-related pathways that precedes clear structural recovery.
- Inflammatory cytokines show partial, power-limited signals:** While IL-1 was significantly increased in the diabetic group, TNF- α showed only a near-significant reduction between diabetic and Retatrutide-only groups, indicating a possible anti-inflammatory trend that may have been underestimated due to reduced sample size and conservative multiple-comparison correction.

5. Renal safety signal and translational implication:

Retatrutide alone did not induce renal toxicity, as total cortical and medullary scores and subparameter scores in the Retatrutide-only group were comparable to controls, aligning with clinical data suggesting a favourable renal safety profile of incretin-based therapies and supporting further evaluation in chronic, T2DM-like models with longer treatment and functional readouts.

ABSTRACT

Purpose

This study investigated whether Retatrutide, a triple incretin agonist, attenuates early diabetes-associated renal injury in a streptozotocin-induced rat model, and whether any protective effect is mediated through modulation of inflammatory cytokines (IL-1, TNF- α) and HIF-1 gene expression in kidney tissue.

Methods

Male Sprague-Dawley rats were divided into four groups: control, STZ-induced diabetic, STZ plus Retatrutide, and Retatrutide alone. Diabetes was induced via a single intraperitoneal STZ injection; Retatrutide was administered subcutaneously for 21 days at a dose adapted from prior metabolic studies. Renal tissue underwent ELISA for interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α), quantitative real-time PCR for HIF-1 expression, and semi-quantitative histopathological scoring of cortical and medullary parameters including haemorrhage, tubular dilatation, inflammatory cell infiltration, tubular vacuolisation, interstitial fibrosis, and intraluminal debris.

Results

STZ-induced rats exhibited significantly elevated cortical and medullary histopathological scores and increased renal IL-1 levels relative to controls, confirming successful early injury induction. Retatrutide did not produce statistically significant histopathological protection, though STZ plus Retatrutide animals showed consistently lower numerical damage scores than STZ-only animals. HIF-1 expression was significantly upregulated in the STZ group and significantly attenuated in both Retatrutide-treated groups. TNF- α showed a non-significant downward trend across comparisons.

Conclusion

Retatrutide selectively modulated hypoxia-related molecular responses in the acute STZ model without conferring overt histopathological renoprotection or inducing intrinsic renal toxicity. These findings indicate that longer treatment durations, refined dosing regimens, and chronic type 2 diabetes models are necessary to determine whether the observed early molecular effects translate into structural renal benefit.

1. INTRODUCTION

Diabetic nephropathy is the most prevalent complication among other complications of diabetes mellitus (DM), and the most debilitating one, since it is one of the major reasons for end-stage renal failure [1]. Progressive decline in the glomerular filtration rate is one of the crucial determinants, as it poses a great risk of forming albuminuria/proteinuria and end-stage renal failure [2]. This condition is not only a reflection of metabolic hyperglycaemia but also of complex cellular and molecular processes, such as oxidative stress, inflammation, TGF- β signalling, and podocyte injury in the renal parenchyma [3].

Diabetic nephropathy occurs in both types of diabetes, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). While it is one of the most prevalent and serious complications of DM, the common feature in the aetiology of diabetic nephropathy is considered to be hyperglycaemia. Although the total number of patients with diabetic nephropathy is higher among individuals with T2DM because of its greater prevalence, age- and duration-adjusted data suggest a stronger association between T1DM and chronic kidney disease and diabetic nephropathy [4–6]. Therefore, we employed the Streptozotocin (STZ)-induced diabetic rat model.

The STZ model is widely used to induce a diabetes-like condition in animals. STZ selectively destroys the pancreatic β -cells and creates a more T1DM-like model [7], which leads to hyperglycaemia-associated early renal injury, including cytokine elevations [8] and hypoxia-induced factor-1 α (HIF-1 α) mediated hypoxic responses, within a relatively short experimental time frame.

Inflammatory responses and tissue hypoxia play a crucial role in the pathogenesis of diabetic renal injury [9]. Disruption of the microvascular structure by the increase of pro-inflammatory cytokines (especially, TNF- α and IL-1 β) in the renal tissue exposed to chronic hyperglycaemia aggravates the glomerular and tubular injury [10,11]. These cytokines contribute to the progression of structural disruptions by promoting the cellular stress responses [10,11]. Additionally, increased oxygen consumption and tissue hypoxia related to impaired perfusion emerge as critical mechanisms that regulate the progressive nature of diabetic nephropathy via HIF-1 α [9].

Current treatment approaches mainly focus on maintaining the glycaemic regulation in decelerating the progression and preventing diabetic nephropathy [12]. However, these strategies seem to be limited in targeting the hypoxic and inflammatory processes that occur in renal tissue [13]. This condition implies that novel pharmacological approaches are needed

because the diabetic renal injury cannot be controlled only by ameliorating the metabolic parameters.

Glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists are one of the agents that are used in the treatment of type 2 DM and obesity and thus are notable for their potential renoprotective effects [14]. Expression of GLP-1R in the renal tissue implies that these agents may exhibit direct effects on glomerular and tubular structures [14]. Finally, previous studies show that GLP-1 agonists may decelerate the progression of diabetic nephropathy by suppressing oxidative stress, inflammation, fibrosis and the cellular processes for apoptosis [14].

By the expansion of knowledge regarding the incretin system, a new generation of agonists was developed, which activate more than one incretin receptor simultaneously [15]. In previous preclinical studies, it was shown that triple receptor agonists, which target the GLP-1, glucose-dependent insulintropic polypeptide (GIP) and glucagon (GCG) receptors altogether exhibit more potent effects on metabolic parameters than dual agonists [16]. Retatrutide (RET) is a triple incretin agonist which activates these three receptors simultaneously and surpasses by its metabolic efficacy in the treatment of obesity and DM [15]. In addition to these, the mechanisms underlying the action of RET have not been fully illuminated. Although the anti-inflammatory and metabolic effects of triple incretin agonists were noted, it is not yet certain whether RET modulates early inflammation and hypoxia-induced pathways directly independent from systemic metabolic recovery.

In this study, we investigated the effects of RET on renal tissue at both histological and molecular levels in the STZ-induced diabetic model. Inflammatory markers associated with diabetic renal injury and hypoxia-related pathways were evaluated. The potential protective effects of RET in early diabetic renal injury were further assessed through quantitative histopathological analysis of renal structural alterations.

2. MATERIAL and METHODS

2.1. Ethical Approval

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals [17] and were approved by the Kütahya Health Sciences University Animal Experiments Local Ethics Committee (Decision No: 2025.06.05, Date: 29.05.2025).

2.2. Chemicals

Retatrutide (RET) was purchased from ALFAGEN Laboratory Equipment Inc. (Türkiye), and Streptozotocin (STZ) was acquired from current laboratory stocks. STZ was dissolved in citrate buffer, and RET in dimethyl sulfoxide (DMSO). To prevent the confounding effects depending on the dissolver, corresponding dissolvers were administered to animals in the control group and related groups (citrate buffer and DMSO, respectively).

2.3. Animals and Accommodation

In our study, 36 Sprague Dawley male rats were employed. Due to the deaths that occurred during the experimental process in the STZ-induced diabetic rats, the number between animals which lived until the end of the experiment and the tissues could be analysed were different. Animals were kept under the conditions of $24 \pm 2^{\circ}\text{C}$ room temperature, $55\% \pm 15$ humidity and a 12 h light/dark cycle and were preserved in suitable standard cages. Water and pellets were provided *ad libitum*. All experimental procedures were performed in the KHSU Experimental Animal Production and Research Application Centre and in the laboratories of the Medical Pharmacology Department, Faculty of Medicine.

2.4. Experimental Design

Rats were separated into four groups randomly: Control (C, $n = 8$), Streptozotocin (S, $n = 5$), Streptozotocin + Retatrutide (SR, $n = 6$), Retatrutide (R, $n = 8$).

For the induction of diabetes, Streptozotocin was administered 60 mg/kg intraperitoneally (i.p.) in citrate buffer. Retatrutide was administered at 0,015 mg/kg dose as indicated before [18] on days between 1-21, subcutaneously in DMSO. To the groups which have not received any drug treatment (Streptozotocin and/or Retatrutide), related dissolvers (citrate buffer or DMSO) were administered in corresponding volumes (2 mL for STZ, 1 mL for RET receiving groups).

Diabetes was confirmed on day 3 by blood glucose measurements (blood glucose > 250 mg/dL). Validation of Streptozotocin-induced diabetes was supported by the provided blood glucose data acquired from our group's previous study [19], which investigated the effects of Retatrutide on learning and memory. In the current study, cognitive and metabolic functions were not re-evaluated. Therefore, analyses in this study were limited to molecular and histopathological examinations performed on the kidney tissue regarding the 3R principle [19].

2.5. Tissue Sampling

Before the sacrifice, rats were weighed. Following this, rats were sacrificed by exsanguination method under ketamine (90 mg/kg) and xylazine (10 mg/kg) anaesthesia. In each animal, one of the kidneys was fixed in the 10% neutral buffered formalin for the histological examination, while the other one was preserved at -80°C for molecular tests.

2.6. Molecular Tests

Renal tissue TNF- α and IL-1 β levels were determined using a solid-phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay) method according to the kit protocol. Measurements were performed at the relevant absorbance wavelength using a ThermoScan microplate reader. The renal tissues kept for the molecular analysis were divided for PCR and ELISA. In six animals (C = 2, S = 1, R = 2), where the supernatant acquired after the homogenisation and centrifugation was not enough for ELISA experiments, cytokine measurement could not be performed. Therefore, ELISA experiments were conducted on 21 animals. All samples were processed as duplicates. The outlier samples, which gave the measurements out of the standard curve of the linear range, were excluded from the analysis. Therewithal, IL-1 β analysis was completed with 19 samples (C = 6, S = 4, R = 4, SR = 5) and TNF- α with 20 (C = 6, S = 4, SR = 5, R = 5) samples.

HIF-1 α gene expression levels were assessed by the quantitative real-time polymerase chain reaction (RT-qPCR) method. Total RNA isolation was performed by using the RNA isolation kit (Promega, USA) according to the manufacturer's instructions. RNA concentration and purity were determined by 260/280 nm absorbance ratio, and RNA integrity was checked via agarose gel electrophoresis. cDNA (Promega, USA) synthesis was performed from the pure RNAs, and the samples were analysed by using the Applied Biosystems StepOnePlus Real-Time PCR system. Amplification specificity was confirmed by melt curve analysis. Ct values were analysed using the $\Delta\Delta C_t$ method, and gene expression changes were calculated using the $2^{-\Delta\Delta C_t}$ formula. GAPDH was used as the housekeeping gene.

2.7. Histopathological Analysis

Kidney tissues were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin blocks. Sections of 4 μ m thickness were cut using a microtome and deparaffinised with xylene, followed by a graded alcohol series. After staining with haematoxylin and eosin

(H&E), the sections were passed through an ascending alcohol series and xylene, then mounted with Entellan. Prepared slides were examined and digitally imaged under a Zeiss Calibri 7 light microscope (Zeiss, Germany). Renal tissues were evaluated for haemorrhage, tubular dilatation, inflammatory cell infiltration, tubular vacuolisation, interstitial fibrosis, and intraluminal debris. In addition, total scoring of cortical and medullary areas was performed. Each parameter was semi-quantitatively scored [20] as follows: 0 = normal tissue, no damage; 1 = mild damage (affected area <25%); 2 = moderate damage (affected area 25–50%); 3 = severe damage (affected area >50%). Two histologists assessed the tissues in a double-blind manner.

2.8. Statistical Analysis

All analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), and graphs were generated using GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, USA). Sample size was a priori calculated using G*Power 3.1.9.7 under the assumptions of a large effect size ($f = 0.40$), $\alpha = 0.05$, and power = 0.80; final group sizes included in the analysis are specified in the relevant table and figure legends. Data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances using Levene’s test. As ELISA-derived IL-1 β levels met assumptions of normality and homogeneity of variance, they were analysed using one-way ANOVA followed by Tukey’s HSD post hoc test; TNF- α levels, for which homogeneity of variance was not met, were analysed using Welch ANOVA followed by the Games–Howell post hoc test. qPCR-derived HIF-1 α expression data and histopathological scores (including cortical and medullary subparameters, as well as total cortical and total medullary scores assigned directly by histologists) showed non-parametric distribution and were, therefore, analysed using the Kruskal–Wallis test followed by Dunn–Bonferroni post hoc test. Parametric data are presented as mean $\pm$ standard error of the mean (SEM), and non-parametric data as median (Q1–Q3); effect sizes are reported as η^2 for ANOVA and η^2_H for Kruskal–Wallis. For all parametric analyses, confidence intervals (CI) were calculated and indicated in the relevant parts of the results section. Statistical significance was set at $p < 0.05$ for all tests.

3. RESULTS

3.1. ELISA Analysis

3.1.1. IL-1 β Levels

IL-1 β levels in kidney tissue were evaluated. One-way analysis of variance revealed a statistically significant difference in IL-1 β levels between the groups ($F(3, 15) = 4.029$, $p = 0.028$) (Figure 1).

According to Tukey's HSD post hoc analysis, IL-1 β levels in the S group were significantly higher than those in the C group (95% CI: 17.4-317.7, $p = 0.026$). No significant differences were observed in the other pairwise comparisons (all $p > 0.05$). Examination of the mean values showed that the highest IL-1 β levels were observed in the S group, followed by the SR and R groups, while the lowest levels were found in the C group (Figure 1).

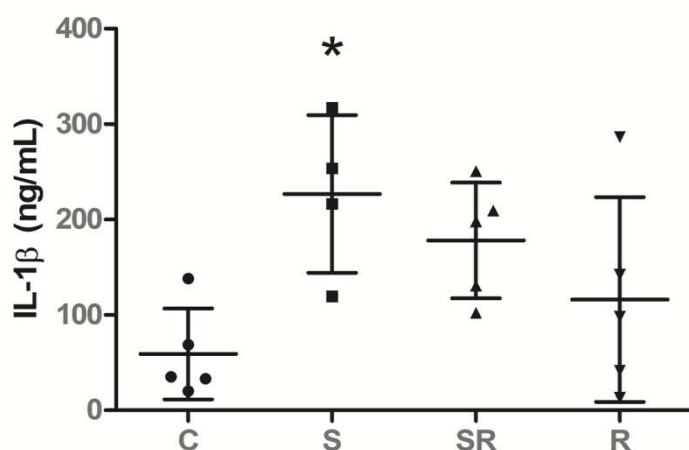


Figure 1: IL-1 β levels. Data are presented as mean $\pm$ standard error of the mean (SEM). * $p < 0.05$ vs. C. Groups: C, control; S, streptozotocin; SR, streptozotocin plus Retatrutide; R, Retatrutide.

3.1.2. TNF- α Levels

TNF- α levels in renal tissue were evaluated. One-way analysis of variance revealed a statistically significant difference in TNF- α levels between the groups ($F(3, 16) = 9.463$, $p = 0.001$). As Levene's test indicated a violation of the homogeneity of variances assumption ($p = 0.006$), Welch ANOVA was performed, which also confirmed a significant difference between the groups ($F(3, 7.776) = 5.301$, $p = 0.028$) (Figure 2).

According to Games–Howell post hoc analysis, no statistically significant differences were detected in pairwise comparisons (all $p > 0.05$); however, the difference between the S and R groups approached statistical significance ($p = 0.083$). Examination of the mean values indicated that TNF- α levels were highest in the S group, while lower levels were observed in the other groups (Figure 2).

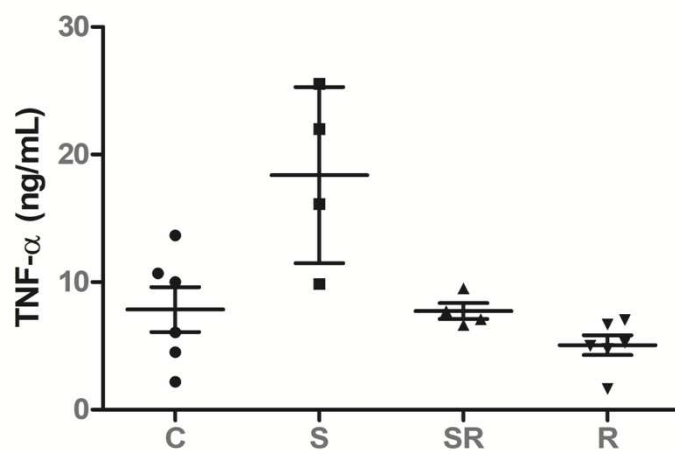


Figure 2: TNF- α levels. Data are presented as mean $\pm$ standard error of the mean (SEM). Groups: C, control; S, streptozotocin; SR, Streptozotocin plus Retatrutide; R, Retatrutide.

3.2. qPCR Analysis

3.2.1. HIF-1 α Gene Expression

HIF-1 α gene expression levels in kidney tissue were evaluated. Kruskal–Wallis analysis revealed a statistically significant difference in HIF-1 α expression between the groups ($H(3) = 9.963$, $p = 0.019$) (Figure 3).

According to Dunn–Bonferroni post hoc analysis, HIF-1 α expression levels in the S group were significantly higher than those in both the SR group ($p = 0.020$) and the R group ($p = 0.048$).

Although the difference between the C and S groups reached significance in the unadjusted comparison ($p = 0.036$), it did not remain significant after Bonferroni correction ($p = 0.214$). No significant differences were observed in the other pairwise comparisons (all $p > 0.05$). Examination of median values indicated that the highest HIF-1 α expression was observed in the S group, while lower levels were found in other groups (Figure 3).

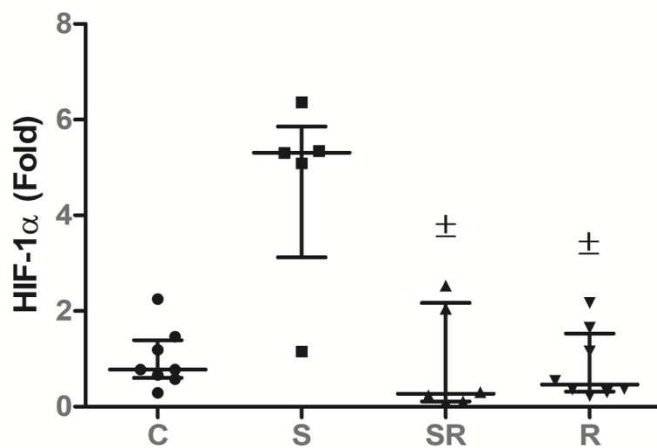


Figure 3: HIF-1 α gene expression levels. Data are presented as median (interquartile range, IQR). $^{\pm}p < 0.05$ vs. S (Dunn–Bonferroni post-hoc). Groups: C, control; S, streptozotocin; SR, streptozotocin plus Retatrutide; R, Retatrutide.

3.3. Histopathological Findings

To evaluate renal histopathological damage, cortical parameters including haemorrhage, tubular dilatation, inflammatory cell infiltration, luminal debris, tubular vacuolisation, and interstitial fibrosis were analysed individually. Kruskal–Wallis analysis revealed statistically significant differences between the groups across all cortical histopathological parameters (all $p < 0.05$) (Table 1, Figure 4).

According to Dunn–Bonferroni post hoc analysis, haemorrhage scores in the S group were significantly higher than those in both the C group ($p = 0.007$) and the R group ($p = 0.020$).

Similarly, inflammatory cell infiltration scores were significantly increased in the S group compared to the C ($p = 0.007$) and R ($p = 0.007$) groups. Tubular dilatation scores were also significantly higher in the S group than in the C ($p = 0.003$) and R ($p = 0.008$) groups. Luminal debris scores were significantly elevated in the S group compared to the C group ($p = 0.019$). Tubular vacuolisation scores were significantly higher in the S group than in both the C ($p = 0.005$) and R ($p = 0.005$) groups. Interstitial fibrosis scores were also significantly increased in the S group compared to the C group ($p = 0.028$). No statistically significant differences were observed in the other pairwise comparisons, as Bonferroni-adjusted p values exceeded the value of 0.05 (Table 1).

Comparison of total cortical histopathological scores using Kruskal–Wallis analysis demonstrated a statistically significant difference between the groups ($H(3) = 14.634$, $p = 0.002$). According to Dunn–Bonferroni post hoc analysis, total cortical scores in the S group were significantly higher than those in both the C group ($p = 0.008$) and the R group ($p = 0.008$). No significant differences were observed in the other comparisons (all $p > 0.05$). Examination of median values indicated that the highest total cortical damage scores were observed in the S group, the SR group exhibited intermediate scores, and lower scores were found in the C and R groups (Figure 4). Representative cortical histopathological images are presented in Figure 5.

Table 1 Median (interquartile range, IQR) values of renal cortical histopathological parameters, Kruskal–Wallis test statistics, and Bonferroni-adjusted Dunn’s post hoc results. Groups: C, control; S, streptozotocin; SR, streptozotocin plus Retatrutide; R, Retatrutide.

Parameters	C median (Q1–Q3)	S median (Q1–Q3)	SR median (Q1–Q3)	R median (Q1–Q3)	H / p (Kruskal–Wallis)	Dunn post-hoc (Bonferroni-adjusted p)
Haemorrhage	0.00 (0.00–1.00)	2.50 (2.00–3.00)	1.50 (0.75–2.00)	1.00 (0.00–1.00)	14.477 / 0.002	S > C ($p = 0.007$); S > R ($p = 0.020$)

Inflammatory infiltration	0.00 (0.00–0.25)	3.00 (3.00–3.00)	1.50 (0.75–2.00)	0.00 (0.00–1.00)	14.284 / 0.003	S > C (p = 0.007); S > R (p = 0.007)
Tubular dilatation	0.00 (0.00–1.00)	3.00 (2.25–3.00)	1.00 (0.75–1.25)	0.00 (0.00–1.00)	16.382 / 0.001	S > C (p = 0.003); S > R (p = 0.008)
Luminal debris	0.00 (0.00–0.25)	1.50 (1.00–2.75)	1.00 (0.00–1.25)	0.00 (0.00–1.00)	10.495 / 0.015	S > C (p = 0.019)
Tubular vacuolization	0.00 (0.00–0.25)	1.50 (1.00–2.00)	1.00 (0.00–1.00)	0.00 (0.00–1.00)	15.164 / 0.002	S > C (p = 0.005); S > R (p = 0.005)
Interstitial fibrosis	0.00 (0.00–0.00)	2.00 (1.25–2.00)	0.50 (0.00–1.25)	0.00 (0.00–0.00)	10.310 / 0.016	S > C (p = 0.028)

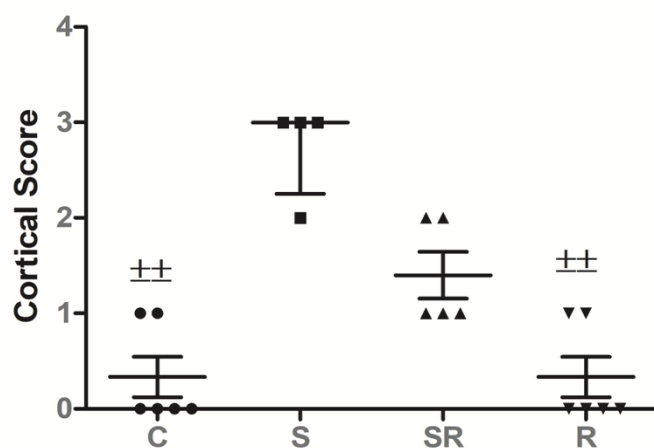


Figure 4: Renal cortical histopathological score. Data are presented as median (interquartile range, IQR). $^{++}p < 0.01$ vs S group (Dunn–Bonferroni post-hoc test). Groups: C, control; S, streptozotocin; SR, streptozotocin plus Retatrutide; R, Retatrutide.

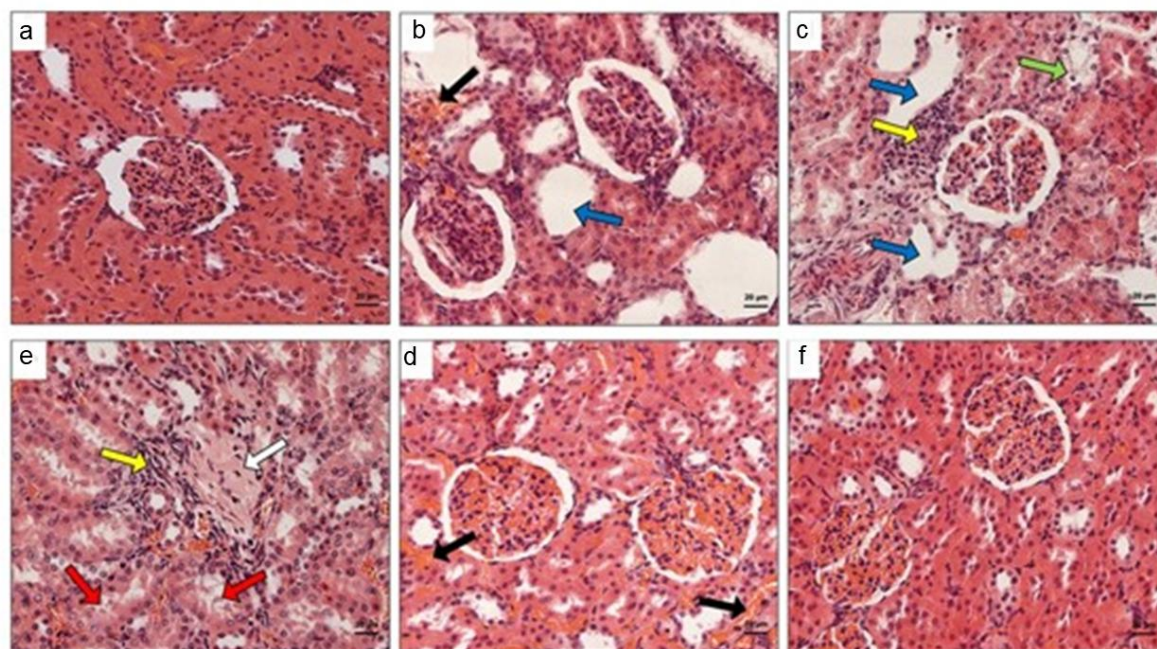


Figure 5: Representative images of the renal cortex. (a) control group; (b–d) streptozotocin group; (e) streptozotocin plus Retatrutide group; (f) Retatrutide group. Black arrow, haemorrhage; blue arrow, tubular dilatation; yellow arrow, interstitial infiltration; green arrow, vacuolisation; white arrow, interstitial fibrosis; red arrow, intraluminal debris. Scale bars, 20 μm .

In the renal medulla, haemorrhage, tubular dilatation, and inflammatory cell infiltration parameters were evaluated. Kruskal–Wallis analysis revealed statistically significant differences between the groups across all medullary histopathological parameters (all $p < 0.01$) (Table 2, Figure 6).

According to Dunn–Bonferroni post hoc analysis, medullary haemorrhage scores were significantly higher in the S group compared to both the C ($p = 0.018$) and R ($p = 0.018$) groups. In addition, haemorrhage scores in the SR group were significantly increased compared to the C ($p = 0.039$) and R ($p = 0.039$) groups. Medullary tubular dilatation scores were significantly higher in the S group than in the C ($p = 0.007$) and R ($p = 0.007$) groups. Similarly,

inflammatory cell infiltration scores were significantly elevated in the S group compared to the C ($p = 0.004$) and R ($p = 0.015$) groups. No statistically significant differences were observed in the other comparisons (all $p > 0.05$) (Table 2).

Comparison of total medullary histopathological scores using Kruskal–Wallis analysis demonstrated a statistically significant difference between the groups ($H(3) = 15.970$, $p = 0.001$). According to Dunn–Bonferroni post hoc analysis, total medullary scores in the S group were significantly higher than those in both the C group ($p = 0.004$) and the R group ($p = 0.011$). No significant differences were observed in the other comparisons (all $p > 0.05$). Examination of median values indicated that the highest total medullary damage scores were observed in the S group, the SR group exhibited intermediate scores, and lower scores were found in the C and R groups (Figure 6). Representative medullary histopathological images are presented in Figure 7.

Table 2 Median (interquartile range, IQR) values of renal medullary histopathological parameters, Kruskal–Wallis test statistics, and Bonferroni-adjusted Dunn’s post hoc results. Groups: C, control; S, streptozotocin; SR, streptozotocin plus Retatrutide; R, Retatrutide.

Figure 6: Renal medullary histopathological score. Data are presented as median (interquartile range, IQR). $^{\pm}p < 0.05$, $^{++}p < 0.01$ vs. S group (Dunn–Bonferroni post-hoc test). Groups: C, control; S, streptozotocin; SR, streptozotocin plus Retatrutide; R, Retatrutide.

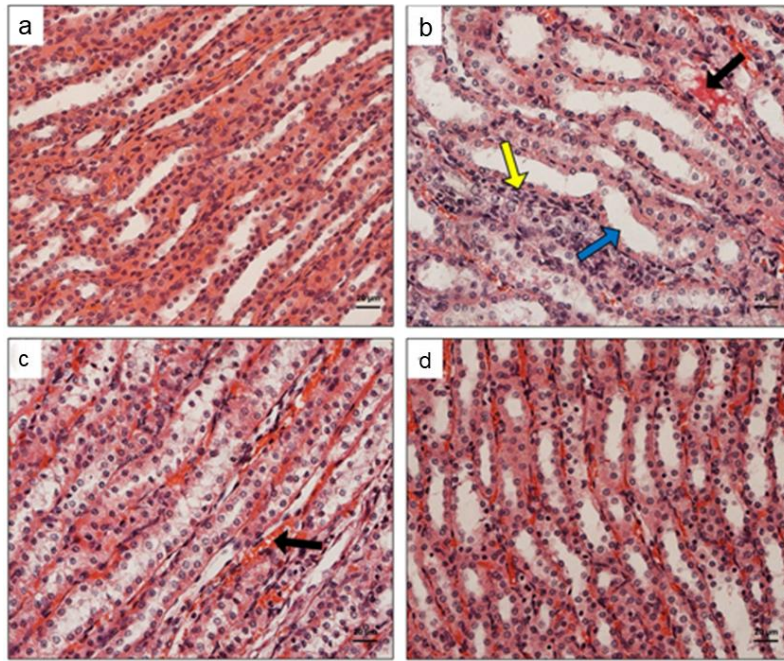


Figure 7: Representative images of the renal medulla. (a) control group; (b) streptozotocin group; (c) streptozotocin plus Retatrutide group; (d) Retatrutide group. Black arrow, haemorrhage; yellow arrow, interstitial infiltration; blue arrow, tubular dilatation. Scale bars, 20 μm .

4. DISCUSSION

To our knowledge, this study is one of the first experimental studies that systematically examines the effects of RET treatment on diabetes-associated renal injury in histopathological (haemorrhage, tubular dilatation, inflammatory infiltration, tubular vacuolisation, interstitial

fibrosis, and intraluminal debris) and molecular (IL-1 β , TNF- α , HIF-1 α) levels altogether. Results acquired reveal that the diabetes-associated renal injury model was successfully established and inflammatory IL-1 β cytokine levels were significantly increased in the S group (STZ-treated) compared to the C group. These results confirm the crucial role of inflammatory response in the pathogenesis of diabetes-associated renal injury and are consistent with previous studies, which have reported an increase in IL-1 β and TNF- α levels [21,22].

In terms of TNF- α levels, notwithstanding that both ANOVA and Welch ANOVA tests have revealed a general significant difference between the groups, in the Games-Howell post-hoc analysis, no pairwise comparison reached statistical significance. Additionally, TNF- α levels were spotted higher in the S group compared to others, and especially the difference between the S and R groups was close to statistical significance ($p = 0.083$). Therefore, it may suggest a tendency toward indicating the important role that this cytokine has in diabetic renal injury. However, within the current sample size and multiple-comparison framework, these differences could not be statistically confirmed. Similarly, in HIF-1 α gene expression, the Kruskal-Wallis test revealed a significant difference between groups ($H(3) = 9.963, p = 0.019$), and by Dunn-Bonferroni post-hoc analysis, it was shown that HIF-1 α levels were significantly higher in the S group compared to the SR ($p = 0.020$) and R ($p = 0.048$) groups. Although the difference between the C and S groups was found to be significant in the initial analysis ($p = 0.036$), this significance was not maintained following correction for multiple comparisons ($p = 0.214$). Nevertheless, the overall HIF-1 α expression profile appears to be consistent with the pattern of hypoxia-associated transcriptional responses observed in diabetic renal injury. Furthermore, the lower HIF-1 α expression observed in the RET-treated groups suggests that RET may modulate molecular pathways related to hypoxia [23,24].

Histopathological evaluation showed that the S group exhibited marked damage across all subparameters in both cortical and medullary regions, whereas in the RET-treated SR group, median scores were numerically lower than those of the S group for all parameters; however, these reductions did not reach statistical significance in Dunn-Bonferroni post hoc analysis (Tables 1 and 2). This pattern suggests that, at the dose and duration tested, RET did not completely reverse diabetic renal injury but produced a limited histopathological improvement in the SR group relative to the S group, characterised by a non-significant trend towards numerical amelioration in several parameters. The dose of 0.015 mg/kg/day used in our study was derived from the range reported by Coskun et al. (2022) [18] as effective for glycaemic

control and weight reduction in obese mouse models, adapted to the present experimental context using the Nair-Jacob mouse-rat allometric conversion [25].

In the cortical region, the S group showed significantly increased scores for haemorrhage, inflammatory cell infiltration, tubular dilatation, and tubular vacuolisation compared with both the C and R groups, whereas for intraluminal debris and interstitial fibrosis, a significant difference was detected only between the S and C groups ($p > 0.05$ for S vs R) (Table 1). The finding that total cortical histopathological scores were significantly higher in the S group than in both the C ($p = 0.008$) and R ($p = 0.008$) groups supports the appropriateness of evaluating cortical structural damage in a multifactorial and cumulative manner. In the SR group, total cortical scores were intermediate between those of the S and C groups (with median values tending towards the C group), indicating a RET-associated trend towards partial histopathological improvement; however, this trend did not reach statistical significance after Bonferroni correction with the current sample size.

In the medullary region, the S group showed significantly higher haemorrhage, tubular dilatation, and inflammatory cell infiltration scores compared with the C and R groups. Notably, medullary haemorrhage scores remained significantly elevated not only in the S group but also in the RET-treated SR group relative to both the C ($p = 0.039$) and R ($p = 0.039$) groups (Table 2). This finding indicates that, although RET treatment brought medullary tubular dilatation and inflammatory infiltration closer to C or R groups' levels, the haemorrhagic component of medullary injury remained a lesion resistant to modification at the tested dose and treatment duration. The observation that total medullary histopathological scores were significantly higher in the S group than in both the C ($p = 0.004$) and R ($p = 0.011$) groups, with the SR group occupying an intermediate position, further suggests that RET treatment was associated with numerical trends towards improvement in some medullary parameters, but these changes did not reach statistical significance.

In contrast, the finding that total cortical and total medullary histopathological scores were significantly higher in the S group than in both the C and R groups indicates that diabetic renal injury is best evaluated not only at the level of individual subparameters, but within an integrated framework that reflects the cumulative impact of multiple structural abnormalities. In both regions, the observation that SR group scores lay between those of the S and C groups suggests a RET-associated trend towards partial histopathological improvement; however, with

the current sample size and the use of a conservative Bonferroni correction for multiple comparisons, this trend did not reach statistical significance.

In phase 2 clinical data, RET has been reported to reduce albuminuria, particularly at higher doses, and to exert favourable effects on eGFR in some cohorts. In this context, the finding of only limited histopathological improvement in the acute STZ model, despite the clinically observed renal benefits in the T2DM/obesity population, is not unexpected, as the STZ model is a standardised experimental system that primarily reflects early structural damage driven by insulin-dependent hyperglycaemia [15,26]. It is generally considered that a substantial proportion of the renal benefit reported in clinical studies arises from metabolic improvements [27,28], such as weight loss and better glycaemic control, which in turn slow the cascade of renal injury [14].

The demonstration that overall group differences detected by the Kruskal–Wallis test for the individual histopathological subparameters were largely driven, in Dunn–Bonferroni analysis, by increased scores in the S group relative to the C and/or R groups supports the methodological appropriateness of using total histopathological scores in the cortical and medullary regions to capture the multifactorial and cumulative nature of diabetic renal injury. The observation that the R group did not differ statistically from the C group in terms of either total cortical and total medullary scores or subparameter scores confirms that RET alone did not induce renal toxicity. This finding is consistent with the favourable renal safety profile of GLP-1 receptor agonists reported in large meta-analyses that include patients with chronic kidney disease [29,30], and supports the view that these agents can generally be continued safely even in the presence of reduced renal function [31].

Our study also has several limitations. First, albeit histopathological assessment and HIF-1 α gene expression analysis were completed in all 27 animals, group sizes for cytokine (TNF- α and IL-1 β) ELISA analyses were reduced to 20 and 19, respectively, due to technical losses during sample preparation and exclusion of samples with readings outside the linear range of the standard curve. This may have partially reduced the statistical power for between-group comparisons of cytokine levels; in particular, the use of conservative multiple-comparison corrections such as Bonferroni increases the risk that true differences fail to reach statistical significance (type II error). Second, HIF-1 α gene expression was measured in whole-renal tissue homogenates, precluding evaluation of potential regional differences between cortical and medullary compartments; moreover, assessment was restricted to mRNA, and changes at

the protein level or in post-translational modifications (hydroxylation, ubiquitination) were beyond the scope of this study. Third, additional important limitations include the fact that the STZ model mimics insulin-dependent hyperglycaemia, that functional parameters such as glomerular filtration rate (GFR) and UACR were not assessed, and that mortality reduced the effective sample size. Fourth, the ordinal structure of the histopathological scores and the stringency of the Bonferroni correction likely limited statistical sensitivity, which may explain why numerical improvements associated with RET in the SR group did not reach significance. These findings should be interpreted alongside the persistence of certain parameters, such as medullary haemorrhage, within this group. Finally, the absence of statistically significant pairwise differences in post hoc analyses for TNF- α , together with the finding that HIF-1 α expression was suppressed in the SR group despite only limited histopathological improvement, suggests that molecular changes may precede structural recovery. This, therefore, indicates that longer treatment durations and/or alternative dosing regimens may be required to achieve overt structural repair. Future studies should incorporate chronic type 2 diabetes models, dose–response protocols, functional renal parameters, fibrosis markers, and immunohistochemical assessment of podocytes and endothelial cells.

5. CONCLUSION

In the STZ-induced diabetic rat model, RET did not exert a statistically significant protective effect on early renal injury at the dose and duration tested; however, it showed a non-significant trend towards improvement, with numerically lower scores than the S group for total cortical and medullary histopathological damage and for the majority of subparameters. In contrast, no evidence of renal toxicity attributable to RET was observed. The robustness of the diabetic model was supported by a significant increase in IL-1 β levels in the S group compared with the C group ($p = 0.026$), as well as by marked increases in histopathological injury across subparameters and in both total cortical ($p = 0.002$) and total medullary ($p = 0.001$) histopathological scores. The finding that RET reduced HIF-1 α expression relative to the S group suggests that it may exert early modulatory effects on hypoxia-related molecular responses. However, the absence of statistically significant histopathological improvement indicates that this molecular effect may not yet have fully translated into overt structural recovery. This implies that longer treatment durations and/or alternative dosing regimens may be required to achieve overt structural repair.

6. DECLARATIONS

Ethics approval:

All experimental procedures were conducted in accordance with the guidelines of the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Kutahya Health Sciences University Animal Research Ethics Committee (Decision No: 2025.06.05, Date: 29.05.2025).

Consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions:

Ulya Keskin and **Melkan Kağan Kara** contributed equally to this work. **Ulya Keskin:** Conceptualisation, Methodology, Formal analysis, Writing -- original draft, Writing -- review and editing, Supervision, Project administration. **Melkan Kağan Kara:** Conceptualisation, Methodology, Formal analysis, Writing -- original draft, Writing -- review and editing. **Eslem Altın, Fikriye Yasemin Özatik, Ayşe Koçak Sezgin, Ayşe Çakır Gündoğdu, Neziha Senem Arı:** Investigation. All authors read and approved the final manuscript.

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- [S1ELISAIL1BetaTNFAlpha.csv](#)