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Review

SS-31 protects diabetic nephropathy progression: A systematic review of *in vivo* and *in vitro* studies

[El SS-31 protege la progresión de la nefropatía diabética: Una revisión sistemática de estudios *in vivo* e *in vitro*]

Jonathan Christianto Sutadji[1#], Dian Anggraini Permatasari Musalim[1#], David Setyo Budi[1#], Jennifer Susanto[1], Fanny Gunawan[1], Chaq El Chaq Zamzam Multazam[2], Citrawati Dyah Kencono Wungu[3,4*]

[1]Faculty of Medicine, Universitas Airlangga, Indonesia.
[2]National Heart and Lung Institute, Imperial College London, London, United Kingdom.
[3]Department of Physiology and Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Indonesia.
[4]Institute of Tropical Disease, Universitas Airlangga, Indonesia.
[#]JCS, DAPM, and DSB are joint first authors.
*E-mail: citrawati.dyah@fk.unair.ac.id

Abstract

Context: Diabetic nephropathy is the leading cause of end-stage renal disease and also death in the world. Administration of Szeto-Schiller-31 (SS-31) as a potential therapeutic candidate that can decrease the renal function damage progressivity in diabetes needs to be comprehensively analyzed.

Aims: To assess the protective effects of SS31 against the progressivity of diabetic nephropathy.

Methods: This systematic review follows PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines 2020. Searches of databases (Pubmed, Science Direct, Scopus, ProQuest, and Springer) were done on 17 September 2023 in order to find articles related to the animal diabetic model and SS-31 treatment. Manual searches from medRxiv were also conducted to obtain additional evidence. Renal function, histopathology analysis, reactive oxygen species *in vivo,* and *in vitro* analysis were described.

Results: There were six *in vivo* studies, each of which discussed the renal function, histopathology, and reactive oxygen species (ROS), and four *in vitro* studies that discussed ROS. The available data suggested that SS-31 improves kidney function by lowering urinary albumin excretion, proteinuria, serum creatinine, creatinine clearance, and BUN, supported by histopathological improvements. In addition, SS-31 also has the effect of lowering 8-hydroxy-2-deoxyguanosine (8-OHdG) level, malondialdehyde (MDA) level, and nicotinamide adenine dinucleotide phosphate (NADPH) expression.

Conclusions: SS31 had a renoprotective effect that could prevent the worsening of renal function in diabetic mice. In addition, the results of histopathology and ROS analysis also support the positive results of SS-31 treatment. Further studies are required to confirm its findings.

Keywords: diabetic nephropathy; elamipretide; mitochondria targeted peptide; SS-31.

Resumen

Contexto: La nefropatía diabética es la principal causa de enfermedad renal terminal y también de muerte en el mundo. Es necesario analizar exhaustivamente la administración de Szeto-Schiller-31 (SS-31) como posible candidato terapéutico capaz de disminuir la progresividad del daño de la función renal en la diabetes.

Objetivos: Evaluar los efectos protectores del SS31 contra la progresividad de la nefropatía diabética.

Métodos: Esta revisión sistemática sigue las directrices PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) 2020. Se realizaron búsquedas en bases de datos (Pubmed, Science Direct, Scopus, ProQuest y Springer) el 17 de septiembre de 2023 para encontrar artículos relacionados con el modelo diabético animal y el tratamiento con SS-31. También se realizaron búsquedas manuales en medRxiv para obtener pruebas adicionales. Se describieron la función renal, el análisis histopatológico, las especies reactivas de oxígeno *in vivo* y el análisis *in vitro*.

Resultados: Hubo seis estudios *in vivo*, cada uno de los cuales analizaba la función renal, la histopatología y las especies reactivas del oxígeno (ROS), y cuatro estudios *in vitro* que analizaban las ROS. Los datos disponibles sugirieron que el SS-31 mejora la función renal al reducir la excreción urinaria de albúmina, la proteinuria, la creatinina sérica, el aclaramiento de creatinina y el BUN, apoyado por mejoras histopatológicas. Además, el SS-31 también tiene el efecto de reducir el nivel de 8-hidroxi-2-deoxiguanosina (8-OHdG), el nivel de malondialdehído (MDA) y la expresión de nicotinamida adenina dinucleótido fosfato (NADPH).

Conclusiones: El SS31 tuvo un efecto renoprotector que pudo prevenir el empeoramiento de la función renal en ratones diabéticos. Además, los resultados de la histopatología y el análisis de ROS también apoyan los resultados positivos del tratamiento con SS-31. Se requieren más estudios para confirmar sus resultados.

Palabras Clave: elamipretida; nefropatía diabética; péptido dirigido a las mitocondrias; SS-31.

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AUTHOR INFO
ORCID: 0009-0001-1595-2528 (JCS) 0000-0001-5180-957X (CDKW)
0009-0004-0206-9084 (DAPM)
0000-0002-0317-6402 (DSB)

INTRODUCTION

Diabetes mellitus is a metabolic disease caused by impaired insulin secretion, insulin work, or both (Chaudhary and Tyagi, 2018). In 2019, 463 million people were reported to have diabetes, which accounted for 9.3% of total global cases (Saeedi et al., 2019). One of its complications is diabetic nephropathy (Chen et al., 2020; Hou et al., 2018; Lim, 2014; Lin et al., 2018). Scholars believe it is the main cause of the patient's morbidity, mortality, and end-stage renal disease (Chen and Chen, 2020; Chen et al., 2020; Lin et al., 2018; Qi et al., 2017). Generally, this complication is caused by prolonged hyperglycemia. Hyperglycemia produces oxidative stress, which can be very harmful, especially for nuclear and mitochondrial DNA (El Baky et al., 2017). It leads to metabolic changes in renal hemodynamics, therefore causing a direct link to podocyte damage, proteinuria, and tubulointerstitial fibrosis, which affect the progression of diabetic nephropathy (Hojs et al., 2020). In addition, mitochondrial involvement and dysfunction might cause reactive oxygen species (ROS) production and ATP reduction, making the damage of diabetic nephropathy more severe (Higgins et al., 2014; Lin et al., 2018). This complication typically manifests in many ways, from albuminuria to overt proteinuria.

Although diabetic nephropathy may harm patients, it can still be cured using several therapies, such as hyperglycemia, hypertension, and dyslipidemia corrections, as well as lifestyle improvements (Lin et al., 2018; McGrath and Edi, 2019). One of the novel medical treatments for diabetic nephropathy is SGLT2-inhibitor (Chen et al., 2020; Garofalo et al., 2019). However, this treatment has several side effects, such as polyuria, genitourinary infection, diabetic ketoacidosis, acute kidney failure, bone fractures, and lower limb amputation (Garofalo et al., 2019; Zoungas and de Boer, 2021). SS-31 (D-Arg-2'6'-dimethylTyr-Lys-Phe-NH2), a member of the Szeto–Schiller (SS) peptide family, is another novel antioxidative peptide targeted on mitochondria. This treatment is reported to inhibit ROS production (Yang et al., 2019b; Zhao et al., 2013) and can scavenge and lessen ROS production. Thus, it can prevent mitochondrial permeability transition and cytochrome c (Cyt c) release (Hou et al., 2016). On the other hand, SS-31 has nephroprotective effects on kidney disease and can decrease renal tubular epithelial cell apoptosis (Wyss et al., 2019; Zhao et al., 2013). SS-31 also has a good safety profile because it has fewer effects on normal mitochondria (Alam et al., 2015).

The novel antioxidant SS-31 has special advantages compared to natural antioxidants (vitamins E, C, and beta-carotene) and mitochondrial-targeted antioxidants (MitoQ and mitochondrial catalase). It works in various ways by serving as antioxidation, apoptosis suppression, mitochondrial quality regulation, and anti-inflammatory properties (Du et al., 2024; Liu et al., 2019). Further, preclinical research has indicated that SS-31 may be able to improve multiple organ systems, such as the skeletal muscles, eyes, brain, heart, and kidneys, by reestablishing mitochondrial function at the cellular level. SS-31 has progressed into a number of clinical trials covering conditions like mitochondrial genetic diseases, mitochondrial myopathy, age-related mitochondrial dysfunction in skeletal muscle, primary mitochondrial diseases, heart failure, myocardial reperfusion injury, renal reperfusion injury, and hereditary corneal diseases. However, the small number of patients included in the study poses limitations. Among the 18 investigations, two studies have not yet finished their trial designs, and the remaining 11 studies have not published the results and related data. As a result, it is currently impossible to precisely analyze the causes of the failure (Du et al., 2024).

As argued earlier, there have been no clinical trials that administer SS-31 in diabetic nephropathy patients. In fact, SS-31 has the potential as an effective therapeutic option to prevent diabetic nephropathy from worsening both *in vivo* and *in vitro* studies. A review that compiles studies investigating the protective effect of SS-31 on the progression of diabetic nephropathy is currently not available. Therefore, this systematic review aims to comprehensively assess the protective effects of SS-31 against the progressivity of diabetic nephropathy in both *in vivo* and *in vitro* studies. It is believed that the administration of SS-31 therapy can improve renal function and histopathology and reduce ROS levels through various pathways and mechanisms.

MATERIAL AND METHODS

This systematic review was conducted based on PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) 2020 guidelines (Page et al., 2021) and had been registered in the PROSPERO database.

Eligibility criteria

This study was a systematic review and included *in vivo* and *in vitro* experimental studies. Specifically, the authors screened the titles and abstracts

independently for eligible studies based on the following criteria: (1) preclinical study included *in vivo* and *in vitro* induced diabetes model ; (2) study involved SS-31 of interest; (3) study reported at least one of outcomes of interest; and (4) they must be writtten in the English language. These criteria generated outcomes such as renal function, renal histopathology, and ROS. On the other hand, some studies were excluded, especially those that came from secondary research (i.e., literature reviews, comments, letters, and editorials), irrelevant articles, and duplications.

Search strategy and selection process

The literature searches were conducted on 17 September 2023 at five databases: Pubmed, Science Direct, Scopus, ProQuest, and Springer. The keywords and booleans used in literature searches were ("diabetes mellitus" OR "diabetic nephropathy") AND ("SS31" OR "Elamipretide" OR "mitochondria targeted peptide" OR "MTP-131"). Having collected the studies from the database, the authors exported them all from the electronic searches manager for duplication removal and screening. The PICO was used to organize the search of this review (populations: animals induced diabetes; intervention: SS-31; comparison: control or placebo; outcomes: renal function, renal histopathology, and ROS). The two review authors (JC and DAPM) independently screened the titles and abstracts of the articles to identify potentially eligible studies, then subsequently screened the full texts. Any disagreements between the two review authors were resolved by discussion with a senior reviewer until a consensus was reached. Excluded studies were described in the PRISMA flow diagram alongside the reasons why they were excluded (Fig. 1).

Data collection process

Two authors (JC and DSB) extracted relevant data from each selected study using structured and standardized forms. The extraction produces essential data, such as (1) study characteristics (author, year of publication, country of origin, study design, group allocation, and number per group; (2) *in vivo* data (species, weight, sex, and age) or *in vitro* data (cells preparation); (3) intervention characteristics (type of intervention from every group dosage, route of administration, and duration of treatment); and (4) outcome data (*in vivo* renal functional outcome, *in vivo* renal histopathology analysis, and *in vivo* and *in vitro* ROS). Any disagreement between the reviewing authors was resolved by discussion until a consensus was reached.

Quality assessment and risk of bias

Before being analyzed, the literature must go through a quality assessment. In this study, authors used SYRCLE's risk of bias tool and CAMARADES checklist to study the quality "Gold Standard Publication Checklist to Improve the Quality of Animal Studies". This checklist was taken from Radboud University Nijmegen Medical Centre and modified by Bule et al. (2020). Research articles with a percentage of quality score ≤ 50 belong to the low-quality group and would be excluded from this study. Any discrepancies were resolved by discussion until a consensus was reached.

RESULTS

Study selections

This investigation began with literature searches from the aforementioned databases and generated 366 articles. These articles were then screened using their titles and abstracts. In this stage, 16 potentially eligible articles were selected to review. After that, the full-text assessment was performed, and six studies were included in this systematic review. The study selection process in this systematic review is illustrated in the PRISMA flow diagram along with the reason for exclusion (Fig. 1).

Quality assessment

The results of SYRCLE's risk of bias tool and CAMARADES checklist for study quality "Gold Standard Publication Checklist to Improve the Quality of Animal Studies" showed that the quality score of the six studies was above 50%. Therefore, they were possible to be included in this study. This finding was similar to previous research that had more than a 50% score. For example, Hou et al. (2016) and Miyamoto et al. (2020) acquired a quality score of 78%, while Hou et al. (2018) and Yang et al. (2019a) had a quality score of 83%. Slightly higher, Wang et al. (2019) scored 89%. Yang et al.'s (2022) finding was recorded as the highest as they managed to obtain a 94% quality score. The quality assessment checklist was summarized in Table S1.

Study characteristics and demographic features

This systematic review found six *in vivo* and four *in vitro* experimental studies with 229 diabetes-induced models that received SS-31 therapy. Five of these were conducted in China (Hou et al., 2016; 2018; Wang et al., 2019; Yang et al., 2019a; 2022), while one was conducted in Japan (Miyamoto et al., 2020) (Table 1).

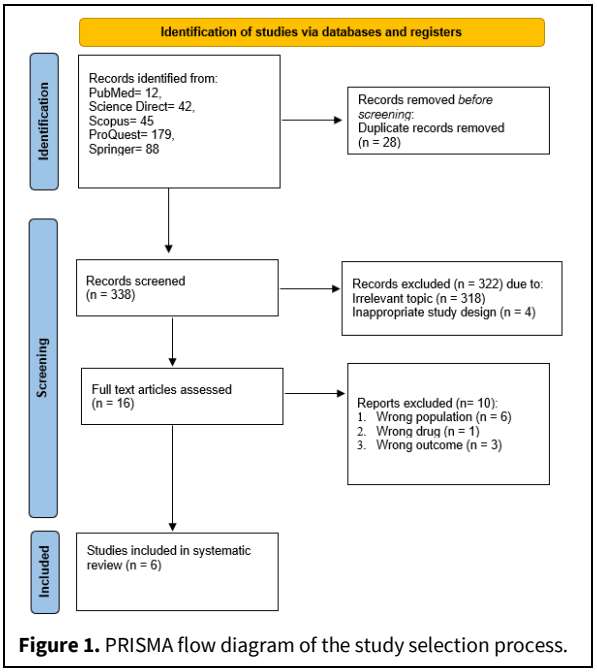

Figure 1. PRISMA flow diagram of the study selection process.

In vivo and *in vitro* characteristics

Based on *in vivo* studies, there were various models of experimental animals, including 8-week-old male C57BLKS/J db/db diabetic and db/m normal male mice (Hou et al., 2018), 40-week-old C57BL/6 mice (Yang et al., 2019a), male Sprague–Dawley (SD) rats (Wang et al., 2019), male CD-1 mice (Hou et al., 2016), male db/db mice (BKS.Cg-Dock7m +/+ Leprdb/J strain) and the corresponding heterozygote lean db/m mice (Miyamoto et al., 2020), and C57BL/6 male mice (Yang et al., 2022). While the diabetic model mice were purchased from the Model Animal Research Center of Nanjing University (Hou et al., 2018) and Jackson Laboratory (Bar Harbor, ME) (Miyamoto et al., 2020), other studies obtained diabetic mice by injecting intraperitoneally streptozotocin 40 mg/kg body weight for five consecutive days (Yang et al., 2019a), streptozotocin 60 mg/kg body weight 16 hours after fasting (Wang et al., 2019), streptozotocin 150 mg/kg body weight (Hou et al. 2016), and streptozotocin 40 mg/kg for three days (Yang et al., 2022). Regarding the SS-31 treatment, the studies administered the SS-31 by giving a dose of 3 mg/kg/day with various duration, including for eight weeks (Hou et al., 2016), 12 weeks (Hou et al., 2018; Miyamoto et al., 2020), 16 weeks (Wang et al., 2019), and 24 weeks (Yang et al., 2019a). Only one study administered SS-31 with a dose of 2 mg/kg/day (Yang et al., 2022) (Table 2).

Based on *in vitro* studies, the cell preparation used in each study varied greatly, including human proximal tubular epithelial cells (HK-2 cells) (Hou et al., 2018; Yang et al., 2019a), podocyte cells (Wang et al., 2019; Yang et al., 2022), and mouse mesangial cells (MMCs; no. CRL-1927) (Hou et al., 2016). The duration of treatment with SS-31 was also different from one study to the others, such as 60 minutes (Wang et al., 2019), 24 hours (Yang et al., 2022), 48 hours (Hou et al., 2016; 2018; Wang et al., 2019), and 72 hours (Yang et al., 2019a) (Table 3).

The effect of SS-31 on kidney function

This study found six studies that reported the effect of SS-31 on kidney function. It was reported that the administration of SS-31 in diabetic mice gave a good outcome on kidney function. Diabetic mice had worse urinary albumin excretion, proteinuria level, serum creatinine, creatinine clearance, BUN, and UACR. They had more significant values than the control group (Hou et al., 2016; 2018; Miyamoto et al., 2020; Wang et al., 2019; Yang et al., 2019a; 2022). The administration of SS-31 was also evidenced to make urinary albumin excretion 5.5 times higher in diabetic mice than in normal ones (Hou et al., 2016). In addition, SS-31 could lower the UAE of diabetic mice with a significant value ($p<0.05$) compared to diabetic mice without SS-31 intervention (Hou et al., 2016; 2018). In addition to reducing albumin secretion, administration of SS-31 could also significantly reduce proteinuria ($p<0.05$) (Wang et al., 2019; Yang et al., 2019a). The increase of serum creatinine, creatinine clearance, and BUN in diabetic mice also decreased significantly with the SS-31 intervention ($p<0.05$) (Hou et al., 2018; Wang et al., 2019; Yang et al., 2019b). In two studies, diabetic mice experienced an increase in UACR, while the administration of SS-31 significantly decreased UACR ($p<0.01$ and $p<0.05$) (Miyamoto et al., 2020; Yang et al., 2022).

Table 1. Overview of the studies.

Author	Country	Sample (N)	Type of controlled laboratory experiments	Animals/cells preparation	Control(s)	Intervention in the main group	Duration of treatment
Hou et al., 2018	China	Experimental animals = 32 (8 in each group)	*In vivo*	Male mice aged 8-week-old C57BLKS/J db/db diabetic and db/m normal male mice	db/db mice were injected with saline intraperitoneally db/m mice were injected with saline intraperitoneally	db/db mice were injected with 3 mg/kg/day SS31 intraperitoneally db/m mice were injected with 3 mg/kg/day SS31 intraperitoneally	12 weeks
			In vitro	High glucose (HG)-induced HK-2 cells. Maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in an atmosphere of 5% CO_2	HK-2 cells were stimulated with 5.6 mmol/L glucose (NG)	After fasting for 24 h, the HK-2 cells were stimulated with: > NG plus 24.4 mM mannitol (M) > 30 mmol/L glucose (HG) > HG plus 100 nM SS31 for 48 hours	48 hours
Yang et al., 2019a	China	Experimental animals = 40 (10 in each group)	*In vivo*	40 eight-week-old C57BL/6 mice (about 20 g body weight)	1st group (control group): Injected with sodium citrate buffer only	2nd group: Mice were injected intraperitoneally with STZ (40 mg/kg body weight) for five consecutive days 3rd group: STZ-induced diabetic mice were injected with normal saline (NS) (5 mL/kg) 4th group: Diabetic mice were intraperitoneally injected with SS31 (3 mg/kg body weight) every other day	24 weeks
			In vitro	Human proximal tubular epithelial cells (HK-2 cells)	N/A	HK-2 cells maintained in > 5 mM D-glucose (LG) > 30 mM D-glucose (HG) > HG plus SS31 (100 nM) > HG plus Mdivi1 (50 μM) > HG medium and normal saline	72 hours
Miyamoto et al., 2020	Japan	Experimental animals = 60 (15 in each group)	*In vivo*	Six weeks of age male db/db mice (BKS.Cg-Dock7m +/+ Leprdb/J strain) and nondiabetic db/m mice	Group 1: db/m mice treated with vehicle (phosphate-buffered saline) (db/m)	Group 2: db/m mice treated with MTP-131 (Bendavia) (db/m+B) Group 3: db/db mice treated with vehicle (db/db) Group 4: db/db mice treated with MTP-131 (db/db+B). MTP-131 (3 mg/kg BW) was subcutaneously injected daily for four weeks and then infused subcutaneously over an 8-week period	12 weeks
			In vitro	N/A			

Table 1. Overview of the studies (continued...)

Author	Country	Sample (N)	Type of controlled laboratory experiments	Animals/cells preparation	Control(s)	Intervention in the main group	Duration of treatment
Wang et al., 2019	China	Experimental animals = 48 (8 in each group)	*In vivo*	Male Sprague–Dawley (SD) rats with an initial weight of 180–220 g were injected intraperitoneally with STZ 60 mg/kg 16 hours after fasting	Group 1: Normal rats injected with PBS Group 2: Normal rats injected with unmodified RSA at 40 mg/kg per day	Group 3: Diabetic rat received iv injection of unmodified RSA at 40 mg/kg per day Group 4: Diabetic rat received iv injection of HOCl-RSA at 40 mg/kg per day Group 5: Diabetic rat received iv injection of unmodified RSA at 40 mg/kg per day and intraperitoneal injection of SS-31 3 mg/kg per day Group 6: Diabetic rat received iv injection of HOCl-RSA at 40 mg/kg per day and intraperitoneal injection of SS-31 at 3 mg/kg per day	16 weeks
			In vitro	Podocyte cells. Podocytes were cultured in 6-well plates at 37°C to differentiate for 12 days	N/A	> 200 mg/L HOCl-MSA for 12, 24, and 48 hours or 200 mg/l native MSA for 48 hours > 1, 10, or 50 nM SS-31 was added 30 minutes before HOCl-MSA for 48 hours > 200 mg/L HOCl-MSA for 15, 30, and 60 min or 200 mg/l native MSA for 60 min > 50 nM SS-31 was added 30 min before HOCl-MSA stimulation for 60 min	48 hours and 60 minutes
Hou et al., 2016	China	Experimental animals = 32 (8 in each group)	*In vivo*	Male CD-1 mice (about 18-22 g body weight). The animal underwent uninephrectomy	Nondiabetic control group (N)	> Nondiabetic group administered SS31 (N + SS31): received 3 mg/kg/day intraperitoneally > Diabetic group administered saline (STZ): received 150 mg/kg STZ intraperitoneally > Diabetic group administered SS31 (STZ+SS31): received 150 mg/kg STZ intraperitoneally and 3 mg/kg/day SS-31 intraperitoneally	8 weeks
			In vitro	Mouse mesangial cells (MMCs; no. CRL-1927)	Cells were stimulated with: > Normal glucose (NG; 5.6 mM) > NG plus mannitol (24.4 mM) as an osmotic control	Cells were stimulated with > HG (30 mM) > NG plus mannitol plus SS31 (100 nM) > HG plus SS31 (100 nM) > HG plus SB203580 (10 M; Promega)	48 hours

Table 1. Overview of the studies (continued...)

Author	Country	Sample (N)	Type of controlled laboratory experiments	Animals/cells preparation	Control(s)	Intervention in the main group	Duration of treatment
Yang et al., 2022	China	Experimental animals = 17 (6 in normal control, 6 in STZ group, 5 in STZ + SS31 group)	*In vivo*	IACUC-1905002 C57BL/6 male mice (weight 18–22 g)	Normal control group: injected with saline intraperitoneally	> Diabetic group (STZ): injected with streptozotocin (STZ) at 40 mg/kg for three days intraperitoneally > Diabetic group administered SS31 (STZ + SS31): injected with SS31 2 mg/kg/day intraperitoneally	4 weeks
			In vitro	Primary podocyte culture seeded in 6-well plates at a density of 1.5×10^5 at 37°C with 5% CO_2	Normal control group	Cells were stimulated with > HG (30 mM) > HG plus SS31 (100 nM) > SS31 (100 nM)	24 hours

HG: high glucose; HOCl-RSA: hypochlorite-modified rat serum albumin; HOCl-MSA: hypochlorite-modified mouse serum albumin; iv: intravenous; N/A: not available; NG: normal glucose; PBS: phosphate-buffered saline; STZ: streptozotocin.

Table 2. *In vivo* study results.

Author	Main outcome measure(s)			Comments	Other outcome measure(s)
	Renal functional	Renal histopathological	ROS		
Hou et al., 2018	1. 24-h UAER (db/db vs. db/m: p<0.01; db/db+SS31 vs. db/db: p<0.05) 2. Scr (db/db vs. db/m: p<0.01; db/db+SS31 vs. db/db: p<0.05)	1. Glomerular hypertrophy (db/db vs. db/m: p<0.01; db/db+SS31 vs. db/db: p<0.05) 2. Tubular injury (db/db vs. db/m: p<0.01; db/db+SS31 vs. db/db: p<0.05)	1. 8-OHdG (db/db vs db/m: p<0.01; db/db+SS31 vs. db/db: p<0.05) 2. MDA level (db/db vs. db/m: p<0.01; db/db+SS31 vs. db/db: p<0.05)	- 24-h UAER and Scr were increased significantly in db/db mice compared with the db/m group. This increase was rescued by the administration of SS31. - The db/db mice group displayed increased glomerular hypertrophy (glomerular volume, mesangial matrix fraction, and glomerular injury index) and tubular injury (proximal tubular area and tubulointerstitial damage) compared with the db/m mice. SS31 treatment reversed these changes. - Urinary MDA and 8-OHdG levels were significantly increased in the db/db group compared with the control db/m group but were reduced by SS31.	SS31 prevented overexpression of CD36, NF-jB (P65), and upregulated MnSOD/CAT inactivation in db/db mice.

Table 2. *In vivo* study results (continued...)

Author	Main outcome measure(s)			Comments	Other outcome measure(s)
	Renal functional	Renal histopathological	ROS		
Yang et al., 2019a	1. Scr (STZ vs. control: 16.82 ± 2.43 vs. 11.99 ± 1.05, p<0.01; STZ+SS31 vs. STZ: 13.62 ± 1.48 vs. 16.82 ± 2.43, p<0.01) 2. BUN (STZ vs. control: 12.98 ± 2.14 vs 9.93 ± 1.48, p<0.01; STZ + SS31 vs STZ: 10.43 ± 1.23 vs. 12.98 ± 2.14, p<0.05) 3. 24 h-Proteinuria (STZ vs. control: 28.41 ± 4.36 vs. 16.00 ± 1.76, p<0.01; STZ + SS31 vs. STZ: 19.91 ± 2.32 vs. 28.41 ± 4.36, p<0.01)	1. Glomerular damage score (STZ vs. control: p<0.01; STZ + SS31 vs. STZ: p<0.01) 2. Tubulointerstitial damage score (STZ vs. control: p<0.01; STZ + SS31 vs. STZ: p<0.01) 3. Renal apoptosis (TUNEL-positive cells/field) (STZ vs. control: p<0.01; STZ + SS31 vs. STZ: p<0.01)	1. Renal MDA concentrations (STZ vs. control: p<0.01; STZ + SS31 vs. STZ: p<0.01) 2. Renal SOD concentrations (STZ vs. control: p<0.01; STZ + SS31 vs. STZ: p<0.01) 3. Renal GSH-PX concentrations (STZ vs. control: p<0.01; STZ + SS31 vs. STZ: p<0.01)	- The levels of serum creatinine, BUN, and microalbuminuria were increased in STZ mice, and SS31 treatment could restore these changes. - Renal glomerular damage, tubulointerstitial damage, and apoptosis were observed in diabetic mice, while SS31 administration could markedly decrease these lesions. - Renal MDA level was increased, while renal SOD and GSH-PX levels were significantly decreased in diabetic mice. SS31 treatment significantly reversed these changes.	SS31 could decrease the expression of Drp1, Bax, caspase1, IL-1β, and FN, while increasing the expression of Mfn1.
Miyamoto et al., 2020	1. Urinary albumin/creatine ratio at 18 weeks (db/db vs. db/m: p<0.001; db/db+B vs db/db: p<0.01)	1. Glomerular hypertrophy (db/db vs. db/m: p<0.001; db/db+B vs. db/db: p>0.05) 2. Mesangial matrix expansion (db/db vs. db/m: p<0.001; db/db+B vs. db/db: p<0.001)	1. DHE oxidation in the glomerulus (db/db vs. db/m: p<0.05; db/db+B vs. db/db: p<0.001), cortical tubules (db/db vs db/m: p<0.001; db/db+B vs. db/db: p<0.01), medulla (db/db vs. db/m: p<0.001; db/db+B vs. db/db: p<0.05) 2. Urinary H_2O_2/creatinine ratio (db/db vs. db/m: p<0.001; db/db+B vs. db/db: p<0.05)	- The urinary albumin/creatinine ratio was significantly increased in the db/db group and significantly suppressed by MTP-131 treatment. - Glomerular hypertrophy and mesangial matrix expansion were observed in diabetic db/db groups. MTP-131 significantly inhibited increases in mesangial matrix accumulation. - The db/db mice have reduced renal superoxide levels as measured by reduced DHE oxidation signal and increased levels of urinary H2O2. Administration of MTP-131 significantly inhibited increases in urinary H_2O_2 and preserved levels of renal superoxide production.	SS31 preserved the expression of LCLAT1, immature CL levels, protection of mitochondrial fusion, reduced total lysoCL levels, the expression of PLA2, and the accumulation of long-chain CL species.

Table 2. *In vivo* study results (continued...)

Author	Main outcome measure(s)			Comments	Other outcome measure(s)
	Renal functional	Renal histopathological	ROS		
Wang et al., 2019	1. Creatinine clearance (G3 vs. G1 and G2, G4 vs. G3: p<0.05; G5 vs. G3 and G4, G6 vs. G3 and G4: p<0.01) 2. Urine protein (G3 vs. G1 and G2, G4 vs. G3: p<0.05; G5 vs. G3 and G4, G6 vs. G3 and G4: p<0.01)	1. The glomerular volume, mesangial area, and basement membrane thickness were significantly increased (G3 vs G1 and G2) and significantly deteriorated (G4 vs G3) in partial tubular epithelial cells. SS-31 prevented renal tissue damage with either native RSA or HOCl-RSA (G5 vs G3 and G4, G6 vs G3 and G4) 2. Podocyte loss (G3 vs G1 and G2: p<0.03; G4 vs G3: p<0.011; G5 vs G3 and G4, G6 vs G3 and G4: p<0.05)	1. HOCl-alb concentrations in plasma (G3 vs. G1 and G2: p<0.05; G4 vs. G3: p<0.007; G5 and G6 vs. G3 and G4: p<0.05) 2. HOCl-alb concentrations in renal tissue (G3 vs. G1 and G2: p<0.05; G4 vs. G3: p<0.004; G5 and G6 vs. G3 and G4: p<0.05) 3. OHdG (G3 vs. G1 and G2: p<0.01; G4 vs. G3: p<0.015; G5 and G6 vs. G3 and G4: p<0.05)	- Urinary protein excretion and creatinine clearance were increased in diabetes rats (Group 3) and significantly deteriorated by HOCl-RSA (Group 4). Administration of SS-31 prevented the increase of proteinuria and creatinine clearance in diabetic rats treated with either native RSA or HOCl-RSA (Groups 5 and 6). The glomerular volume, mesangial area, basement membrane thickness, and podocyte loss were significantly increased in diabetic rats (Group 3) and significantly deteriorated by HOCl-RSA challenge in diabetic rats (Group 4). Administration of SS-31 prevented renal tissue damage (Group 5 and Group 6). - The plasma and renal HOCl-alb concentrations and urinary 8-OHdG were spontaneously elevated in diabetic rats (Group 3), and enhanced in the HOCl-RSA-treated diabetic rats. SS31 significantly restored the increase of the HOCl-alb concentration in both plasma and renal tissues of diabetic rats.	SS-31 restored the increase of the plasma and renal tissues HOCl-alb concentration, cyt c in cytoplasm and reduction of cyt c in mitochondria. The upregulation of cleaved fragments of caspase-3, caspase-7, and PARP-1.
Hou et al., 2016	1. 24-h UAE (STZ vs. N: p<0.01; STZ+SS31 vs. STZ: p<0.05)	1. Glomerular hypertrophy (STZ vs. N: p<0.01; STZ+SS31 vs. STZ: p<0.05) 2. Glomerular injury index (STZ vs. N: p<0.01; STZ+SS31 vs. STZ: p<0.05) 3. Apoptotic cells (STZ vs. N: p<0.01; STZ+SS31 vs. STZ: p<0.05)	1) 8-OHdG (STZ vs. STZ+SS31: p<0.05); STZ vs. N+SS31: p<0.01) 2) NADPH Oxidase (HG vs. HG+SS31: p<0.05)	- UAE was about 5.5-fold higher in the STZ group compared with the N group, and SS31 treatment significantly alleviated diabetic mice UAE. - Glomerular hypertrophy and glomerular injury index were more prominent in STZ Group compared with N Group mice, which was ameliorated by SS-31 treatment. - Urinary 8-OHdG level and NADPH oxidase activity were increased in the STZ group than that of the N group, and SS31 treatment significantly reduced 8-OHdG excretion and suppressed the increase of NADPH oxidase activity.	SS-31 treatment reduced the number of apoptotic cells, inhibited the increased expression of Bax protein and mRNA, TGF- 1, TXNIP protein and mRNA, p-p38 MAPK, p-CREB, nox4 protein, and promoted the expression of Bcl-2 protein and mRNA.

Table 2. *In vivo* study results (continued...)

Author	Main outcome measure(s)			Comments	Other outcome measure(s)
	Renal functional	Renal histopathological	ROS		
Yang et al., 2022	1) Urinary albumin-creatine ratio (STZ vs. Ctrl: p<0.05; STZ + SS31 vs. STZ: p<0.05)	1) Mesangial matrix expansion (STZ vs. Ctrl: p<0.05; STZ + SS31 vs. STZ: p<0.05) 2) Loss of podocyte (STZ vs. Ctrl: p<0.01; STZ + SS31 vs. STZ: p<0.01) 3) Foot process fusion (STZ vs. Ctrl: p<0.01; STZ + SS31 vs. STZ: p<0.05)	N/A	- Microalbuminuria was increased in the STZ group, and SS31 significantly reduced albuminuria in diabetic mice. - The STZ group showed significant mesangial matrix expansion, loss of podocytes, and foot process fusion, while SS31 rescued these changes.	SS31 reserved nephrin downregulation, increased mitochondria number and preserved mitochondrial cristae sharp, ameliorated mitochondrial fragmentation, could halt the downregulation of OPA1 in glomeruli, and inhibited the activation of OMA1.

8-OHdG: 8-hydroxydeoxyguanosine; B: bendavia; BUN: blood urea nitrogen; CL: cardiolipins; DHE: dihydroethidium; FN: fibronectin; GSH-PX: glutathione peroxidase; H2O2: hydrogen peroxidase; LCLAT1: lysocardiolipin acyltransferase 1; MDA: malondialdehyde; MnSOD/CAT: Mn superoxide dismutase and catalase; Pla2: phospholipase A2; Scr: serum creatinine; SOD: superoxide dismutase; STZ: streptozotocin; UAER: urinary albumin excretion rate; Ctrl: control; G1: Group 1; G2: Group 2; G3: Group 3; G4: Group 4; G5: Group 5; G6: Group 6; HOCl-RSA: hypochlorite modified rat serum albumin; HOCl-alb: hypochlorite-modified albumin; N/A: not available; OHdG: hydroxydeoxyguanosine; cyt c: cytochrome c; UAE: urinary albumin excretion; STZ: streptozotocin.

Table 3. *In vitro* study results.

Author	Main outcome measures (ROS)	Comments	Other outcome measure(s)
Hou et al., 2018	Mitochondrial SOX median fluorescence intensity (HG vs. NG: p<0.01; HG+SS31 vs. HG: p<0.05)	In HK-2 cells, the fluorescence intensity of ROS production was significantly enhanced after stimulation with HG compared with the control group. This increase was reduced markedly in cells co-incubated with SS31.	SS31 increased MnSOD and CAT levels, reduced NADPH oxidase activity, suppressed NADPH oxidase subunits, CD36 and NF-jB p65, and activated MnSOD and CAT in HG-induced HK-2 cells.
Yang et al., 2019a	Relative mitochondrial ROS (HG vs LG: p<0.01; HG+SS31 vs HG: p<0.01)	Mitochondrial ROS levels were increased in HK-2 cells under an HG environment; these changes were reversed in cells pretreated with SS31.	Pretreatment with SS31 could downregulate the expressions of Drp1, Bax, Caspase1, IL-1β, and FN in HK-2 cells under high-glucose conditions.
Miyamoto et al., 2020	N/A		
Wang et al., 2019	Intracelluler ROS (HOCL-MSA 60 min vs MSA: p<0.05; HOCL-MSA+SS31 vs HOCL-MSA 60 min: p < .0001)	Incubation of 200 mg/L HOCl-MSA with podocytes for 15, 30, and 60 min induced an increase of intracellular ROS compared with native MSA-treated cells, and treatment with 50 nM SS-31 reversed the increase of intracellular ROS induced by HOCl-MSA stimulation for 60 min.	SS-31 inhibited HOCl-MSA-induced podocyte apoptosis and prevented mitochondria-dependent apoptosis signaling by HOCl-alb as evidenced by the release of cytochrome c (cyt c), binding of Apaf-1 and caspase-9, and activation of caspases.
Hou et al., 2016	Intracellular ROS (HG vs NG: p<0.01; HG+SS31 vs HG: p<0.05)	The intracellular ROS production increased significantly in the HG group, which was remarkably suppressed with SS-31 treatment.	SS-31 inhibited HG-mediated apoptosis, expression of cleaved caspase-3, Bax/Bcl-2 ratio, and cytochrome c (cyt c), normalized the expression of TGF-1, Nox4, and TXNIP, and activation of p38 MAPK and CREB and NADPH oxidase activity.
Yang et al., 2022	N/A		

Apaf-1: apoptosis activated factor 1; CAT: catalase; FN: fibronectin; HG: high glucose; HOCl-alb: hypochlorite-modified albumin; HOCl-MSA: hypochlorite modified mouse serum albumin; LG: low glucose; MnSOD/CAT: Mn superoxide dismutase; N/A: not available; NADPH: nicotinamide adenine dinucleotide phosphate; NG: normal glucose; ROS: reactive oxygen species; TGF: transforming growth factor; TXNIP: thioredoxin-interacting protein.

Effect of SS-31 on renal histopathology

There were six studies that reported the results of renal histopathology after being administered SS-3 by protecting diabetic mice kidneys against pathological damage. Studies in this systematic review indicated that diabetic mice displayed glomerular damage and hypertrophy, mesangial matrix expansion, and tubulointerstitial damage. Additionally, the mice had a significant increase of apoptotic cells compared to the control group (Hou et al., 2016; 2018; Miyamoto et al., 2020; Wang et al., 2019; Yang et al., 2019a; 2022). The studies also highlighted the characteristics of the glomerulus in diabetic mice where there was an increase, for example, in glomerular size (Hou et al., 2016; 2018; Miyamoto et al., 2020; Wang et al., 2019), mesangial matrix expansion (Miyamoto et al., 2020; Yang et al., 2022), glomerular injury index (Hou et al., 2016; Yang et al., 2019a), and loss of podocyte (Wang et al., 2019). Strikingly, SS-31 administration could significantly relieve these issues (Hou et al., 2016; 2018; Miyamoto et al., 2020; Wang et al., 2019; Yang et al., 2019a; 2022). Only one study showed a slight improvement in glomerular size after SS-31 administration, but it was not significant compared to diabetic mice (Miyamoto et al., 2020). The tubulointerstitial damage in diabetic mice was also reversed by SS-31 administration, as shown in several studies like Hou et al. (2018) and Yang et al. (2019a). In addition, they reported that SS-31 could significantly alleviate apoptotic cells in renal cortical tubules (Hou et al., 2016; Yang et al., 2019a).

Effect of SS-31 on reactive oxygen species

This systematic review found five studies *in vivo* and four *in vitro* that reported the effects of SS-31 administration on ROS. One of the effects was regarding the urinary 8-OHdG level, which significantly increased in the diabetic mice group. Interestingly, it was proven that administering SS-31 to diabetic mice might decrease the 8-OHdG level compared to those without SS-31 administration (p<0.05) (Hou et al., 2016; Hou et al., 2018; Wang et al., 2019). SS-31 administration was also reported to significantly lower the MDA level (p<0.05 and

p<0.01) that increased in the diabetic group (Hou et al., 2018; Yang et al., 2019a). Moreover, the effect of SS-31 was reported to suppress the increase in urinary H_2O_2 (p<0.05) (Miyamoto et al., 2020), plasma and renal HOCl-alb concentration (p<0.05) (Wang et al., 2019), and NADPH oxidase activity (p<0.05) (Hou et al., 2016). On the other hand, all the *in vitro* studies showed that ROS production enhanced significantly under an HG environment, and these changes were reduced markedly after undergoing an SS-31 treatment (Hou et al., 2016; 2018; Wang et al., 2019; Yang et al., 2019a).

DISCUSSION

This study reviewed studies that assessed the protective effects of SS-31 toward diabetic nephropathy in *in vivo* and *in vitro* studies. The reviewed studies reported that diabetic mice suffered kidney damage with increased urinary albumin excretion, proteinuria levels, serum creatinine, BUN, and UACR. In addition, the histopathology of renal cells in diabetic mice showed glomerular damage and hypertrophy, tubulointerstitial damage, and increased apoptotic cells (Hou et al., 2016; Hou et al., 2018; Miyamoto et al., 2020; Wang et al., 2019; Yang et al., 2019a; 2022). Those damages are often associated with hyperglycemia that causes afferent arteriolar dilation through the release of vasoactive mediators, such as insulin-like growth factor 1 (IGF-1), glucagon, nitric oxide (NO), vascular endothelial growth factor (VEGF), and prostaglandin.

The presence of hyperglycemia and insulin resistance can cause endothelial dysfunction by increasing ROS production, protein kinase c (PKCs) activation, and advanced glycation end-products (AGE)-induced proinflammatory signaling. In addition, hyperglycemia can produce electron donors Nicotinamide Adenine Dinucleotide (NADH) and Flavin Adenine Dinucleotide (FADH2), which suppress the ability of mitochondria to carry out electron transport to form superoxide and several other ROS. As a result, it damages DNA and reduces the activity of the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Inactivation of this enzyme causes the accumulation of glycolytic products, which then will be converted into harmful metabolite products, resulting in hyperfiltration, endothelial injury, microvascular disease, and albuminuria (Lin et al., 2018).

In people with diabetes, persistent hyperglycemia can increase ROS and eventually end in the death of pancreatic β cells (Qadarsih et al., 2022). The phospholipid barrier, proteins, lipids, and nucleic acid will all be destroyed by chronic hyperglycemia, which will also trigger cell death and the apoptotic process (Al-Aubaidy and Jelinek, 2010; Li et al., 2011). High glucose levels are associated with increased hydrogen peroxide (H_2O_2) emission at complex III and mitochondrial injury in diabetes mellitus (Ding et al., 2021). There has been convincing evidence about free radicals and oxidative stress in diabetes mellitus pathogenesis and complications, such as diabetic nephropathy (Pasupuleti et al., 2020). In diabetic nephropathy, mitochondria are considered a large ROS producer and become the target of oxidative damage (Li et al., 2011; Lin et al., 2018). Diabetic nephropathy is associated with decreased mitochondrial superoxide, causing a decrease in AMPK activity and eNOS phosphorylation. The decrease stimulates the inflammatory process and vascular dysfunction (Lin et al., 2018). In addition, mitochondrial dysfunction and ROS production will trigger cell apoptosis.

This systematic review promotes the use of SS-31 treatment since it selectively targets the mitochondrial inner membrane and prevents oxidative stress by enhancing oxidative phosphorylation coupling. By so doing, ATP production can be improved, and mitochondrial ROS generation is reduced (Li et al., 2011; Wyss et al., 2019). As the primary source of ROS production, SS-31 will be localized in the inner membrane of the mitochondria, where it will also reduce mitochondrial permeability by inhibiting the accumulation of positive molecules (Ding et al., 2021). SS-31 also controls organ damage mediated by the NF-κB pathway (Wyss et al., 2019). According to Li et al. (2011), SS-31 administered to isolated mitochondria can reduce H_2O_2 formation, increase O_2 consumption, increase ATP production, and inhibit lipid peroxidation.

The main action of SS-31 as an antioxidant is to scavenge H_2O_2, ONOO, and also dimethyl tyrosine residue (Escribano-López et al., 2019). When interacting with oxyradicals, tyrosine residue in SS peptide can form unreactive tyrosine radicals. Meanwhile, tyrosine radicals combined with other tyrosine will form dityrosine. As residue from dityrosine, dimethyltyrosine or the phenolic compound 3,5-dimethylphenol contributes to inhibiting oxidative stress and lipid peroxidation (Ding et al., 2021). Leukocyte-endothelial interaction, oxidative stress, and inflammation can all be decreased in T2 diabetes patients by SS-31. It can also raise SIRT1 in leukocytes (Escribano-López et al., 2019). SS-31 injection (5 mg/kg) four times in four weeks can decrease the hyperglycemia-induced elevation of H_2O_2, enhance fusion-related mRNA and protein levels, decrease fission activity in the liver, alleviate lipid peroxidation, and normalize the architecture of pancreatic β-cells (Ding et al., 2021).

In the kidney, lipotoxicity-induced ER stress can be reduced by SS-31. Within four weeks, SS-31 has a favorable effect on the kidneys. Research findings indicate that over four weeks, there was a decrease in both the albumin-to-creatinine ratio and the serum creatinine level, which was accompanied by a decrease in podocyte loss (Ding et al., 2021). Based on this review, SS-31 mechanisms in preventing diabetic nephropathy progression are manifested in many ways. For example, it inhibits TXNIP and Nox4 expression, p38 MAPK activation, and CREB and NADPH oxidase activity in diabetic kidney and mesangial cells (Hou et al., 2016). In addition, it normalizes lysoCL via restoration of LCLAT1 expression (Miyamoto et al., 2020), decreases mitochondrial fragmentation by suppressing the expression of Drp1, increases the expression of Mfn1 (Yang et al., 2019a), and inhibits CD36-triggered lipid accumulation (Hou et al., 2018).

Likewise, this systematic review found that SS-31 improves kidney function and renal histopathology of diabetic mice. SS-31 administration to diabetic mice was evidenced to lower UAE, proteinuria, serum creatinine, creatinine clearance, and BUN levels compared to those in the control group (Hou et al., 2016; 2018; Miyamoto et al., 2020; Wang et al., 2019; Yang et al., 2019a; 2022). The histopathological analysis also indicated that administering SS-31 to diabetic mice decreased glomerular damage and glomerular injury index, renal tubulointerstitial injury, and cell apoptosis. It also prevented podocyte damage. In order to support the results of *in vivo* studies, *in vitro* studies performing ROS analysis were reviewed. The results showed that 8-OHdG and MDA levels decreased after receiving the SS-31 treatment.

Red blood cells have a cellular antioxidant called erythrocyte-reduced glutathione (GSH). In diabetes patients, the glutathione pool is smaller and more oxidized than in the control group, weakening the defense against oxidative stress. Erythrocytes in diabetes are more susceptible to lipid peroxidation. Lipid peroxidation can be measured by malondialdehyde (MDA) in a reaction to thiobarbituric acid–reactive substances (TBARS) (Al-Aubaidy and Jelinek, 2010). Free radicals in diabetes mellitus cause the accumulation of malondialdehyde (MDA) by a peroxidative breakdown of phospholipids (El Baky et al., 2017). MDA is a lipid peroxidation marker that is often used in patients with renal disease. In several studies, it was found that MDA serum levels of patients with chronic kidney disease were higher than in healthy people. Additionally, MDA was negatively correlated with the glomerular filtration rate (Hojs et al., 2020). Meanwhile, the measurement of 8-OHdG is useful for evaluating DNA damage in the whole body. Cytosine and guanine are found in the body. Guanine is more vulnerable to DNA damage because it has a lower redox potential. Guanine in DNA will interact with cytosine, which is called guanosine. Deoxyguanosine (dG) undergoes an oxidation reaction, then the hydroxyl radical (OH) will occupy the carbon-8 (C-8) position in the guanine base, which will then become 8-hydroxy-2'-deoxyguanosine (8-OhDG) (Qadarsih et al., 2022). 8-OHdG levels are elevated in diabetic patients and reported as a biomarker of early diabetic complications stage (El Baky et al., 2017). Elevations of 8-OHdG in serum and urine were reported to be higher in diabetic patients with complications of DKD. Patients with higher urinary 8-OHdG levels had worsened progression of DKD. Thus, urinary 8-OHdG was the strongest predictor of nephropathy (Hojs et al., 2020). Furthermore, elevated 8-OHdG in prediabetic individuals is not only a significant marker of oxidative damage but also predisposes them to subclinical microvascular damage, particularly retinopathy and nephropathy (Al-Aubaidy and Jelinek, 2010).

This is the first systematic review that examines the SS-31 possible effects in diabetes nephropathy as a mitochondrial-targeted peptide. Despite its novel findings, this study has several limitations. First, it indeed has good results in the mechanism of preventing the progression of diabetic nephropathy. However, the most effective length of treatment is not yet known in detail. Second, the six *in vivo* and *in vitro* studies did not evaluate adverse events and toxicity, which are important factors in a treatment process. However, the RCT studies regarding the administration of SS-31 to genetic disorders of mitochondrial cardiolipin metabolism reported 12/12 (100%) occurrences of at least one adverse event after SS-31 administration more than 10/12 placebo (83.3%). The majority of adverse events were injection pain, including erythema at the injection site (12/12 elamipretide vs. 3/12 placebo), bronchitis (2/12 elamipretide vs. 1/12 placebo), and headache (1/12 elamipretide vs. 3/12 placebo). The RCT study indicates that the SS-31 treatment is safe enough because the majority of adverse events are mild-moderate degrees (Thompson et al., 2021). Unfortunately, the long-term effect of therapy is still unknown due to limited studies. Third, this study only discusses the therapeutic effect on *in vivo* and *in vitro* studies. Therefore, the effect on humans is still uncertain. Moreover, the number of studies included in this systematic review is very small. Thus, sample size can be one of the primary considerations for researchers who would like to investigate this topic in the future.

CONCLUSION

This systematic review demonstrates that SS-31 has a renoprotective effect that can prevent the worsening of renal function in diabetic mice. This finding is confirmed by histopathology and reactive oxygen species analysis. The results of *in vitro* and *in vivo* experiments support SS-31 potential as a candidate for clinical trials in diabetic nephropathy. However, several limitations in this study can encourage further investigations to carefully examine other issues, such as effective dose, length of treatment, and adverse events.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Sutadji JC	Musalim DAP	Budi DS	Susanto J	Gunawan F	Multazam CEZ	Wungu CDK
Concepts or ideas	x	x	x				
Design	x	x	x				x
Definition of intellectual content						x	x
Literature search	x	x	x	x	x	x	x
Experimental studies	x	x	x				
Data acquisition							x
Data analysis	x	x	x	x	x	x	x
Statistical analysis	x	x	x				
Manuscript preparation	x	x	x	x	x	x	x
Manuscript editing	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x

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Supplementary data.

Table S1. Quality Assessment using the SYRCLE's risk of bias tool and CAMARADES checklist for study quality "Gold Standard Publication Checklist to Improve the Quality of Animal Studies."

Question	Hou et al. (2016)	Hou et al. (2018)	Miyamoto et al. (2020)	Wang et al. (2019)	Yang S et al. (2019a)	Wang et al. (2022)
Research question specified and clear?	1	1	1	1	1	1
Method of allocation to a treatment group: i.e., Animals randomized across groups?	1	1	1	1	1	1
Sample-size calculation before the start of the experiment	0	0	0	0	0	0
Concealment of allocation?	1	1	1	1	1	1
Group characteristics clearly described?	1	1	1	1	1	1
Correct control group used?	1	1	1	1	1	1
Is body temperature controlled?	0	1	0	1	0	0
Number of animals per group clear?	1	1	1	1	1	1
Age, sex, and weight of the animal	1	1	1	1	1	1
Complete outcome data?	1	1	1	1	1	1