Saudi Journal of Biological Sciences 30 (2023) 103720

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Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Lepidium sativum alleviates diabetic nephropathy in a rat model by attenuating glucose levels, oxidative stress, and inflammation with concomitant suppression of TGF-β1

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ARTICLE INFO

Article history:
Received 2 June 2023
Revised 15 June 2023
Accepted 23 June 2023
Available online 28 June 2023

Keywords:
Lepidium sativum
Type 1 diabetes
Nephropathy
Inflammation
Rats

ABSTRACT

In this research, the treatment of diabetic nephropathy in rats induced by streptozotocin with *L. sativium* whole-plant aqueous extract was examined, and the mechanism of action was proposed. Adult male rats were grouped into: control, *L. sativum*, T1DM, and T1DM + *L. sativum*-treated groups. For 8 weeks, *L. sativum* S was given to rats at a final dose of 250 mg/kg. Treatment with *L. sativum* reduced the amount of fasting glucose, increased the amount of fasting insulin, and diminished the increase in hepatic and serum cholesterol, free fatty acid, and triglyceride levels. The level of serum LDL-c was reduced. At the level of the kidney, *L. sativum* reduced urine volume and albumin excretion and spiked creatinine excretion. It also attenuated the tubular damage in the rats' kidneys and reduced the amounts of major inflammatory markers, including nuclear factor-kappaα (NF-κB), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). Interestingly, *L. sativium* reduced the amount of mRNA transforming growth factor-β1 (TGF-β1), stimulated mRNA superoxide dismutase (SOD) and catalase (CAT), reduced lipid peroxide levels (MDA), and increased the glutathione (GSH), SOD, and CAT in the rat kidneys of the control and T1DM-treated group. In conclusion, *L. sativum* is a novel therapy against DN owing to its hypoglycemic effect, insulin-releasing, and antioxidant potential.

1. Introduction

Diabetes mellitus (DM) is a worldwide spread metabolic disorder resulting from either a defect in insulin release (T1DM) or (T2DM) and is related to the disturbance of protein, carbohydrate, and fat metabolism (Sadri et al., 2017). The disease is also associated with several microvascular and macrovascular clinical complications, representing severe socioeconomic burdens and an increased morbidity and mortality rate (Viigimaa et al., 2020). Diabetic nephropathy (DN) is a common complication that appears early in people with type 1 DM, which is characterized by hyperfiltration and microalbuminuria. However, later symptoms include a decreased glomerulus filtration rate (GFR), persistent microalbuminuria, and severe proteinuria, which can develop into end-stage renal disease (ESRD), fibrosis, and renal dysfunction (Ricciardi and Gnudi, 2021).

Currently, a number of variables are thought to contribute to the development of DN, including hyperglycemia, hyperlipidemia, and hypertension (Kanwar et al., 2011; Ricciardi and Gnudi, 2021). In addition, the etiology of DN points to inflammation, oxidative stress, and apoptosis as the main molecular pathways influencing kidney health in human and animal models with diabetic (Kanwar et al., 2011; Mima,2013; Sha et al., 2017). Indeed, it was shown that hyperglycemia-induced oxidative stress, rather than other hemodynamic or metabolic factors, is a key mechanism that can induce renal damage by provoking inflammation, fibrosis, and apoptosis (Sifuentes-Franco et al., 2018). However, reducing oxidative stress by antioxidants or overexpressing endogenous antioxidant factors protects against experimentally induced DN

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2023.103720
1319-562X/

by alleviating all the above-associated damaging pathways (Koya et al., 2003; Mima, 2013; Sagoo et al., 2018). In addition, the available data have shown that hyperglycemia can promote oxidative stress in kidneys by producing a lot of reactive oxygen species (ROS) and depleting endogenous antioxidants by activating several dangerous mechanisms/routes (Kanwar et al., 2011; Sagoo et al., 2018; Mima, 2013). These include glucose autoxidation, protein glycation, ROS-generating pathways activation, and the stimulation of several mitochondrial ROS-generating enzymes (i.e., lipoxygenases, xanthine oxidase, nitric oxide synthases) (Kanwar et al., 2011).

In contrast, current data have also pointed to the importance of growth factors in hyperglycemia-induced DN in diabetic animals (Schena and Gesualdo, 2005; Kanwar et al., 2011; Voelker et al., 2017). In general, TGF-β1 is a major cytokine that controls a variety of biological processes like cell division, differentiation, migration, adipogenesis, and osteogenesis and is produced by cells in many organs, such as kidneys (Voelker et al., 2017). The kidneys of diabetic people and animals have significantly higher amounts of TGF-1 protein and mRNA, which are linked to the disease's severity, stage, and symptoms (Zhao et al., 2020). From this view, hyperglycemia, PKC, angiotensin II, and mechanical stress are potent stimulators of TGF-β1 during T1DM (Chang et al., 2016; Zhao et al., 2020). The pathological renal consequences for the renal activation of this factor include a significant increase in glucose consumption resulting from the upregulation of GLUT-1 mRNA and protein levels (Zhao et al., 2020). Furthermore, TGF-β1 is the main fibrotic agent that causes renal cell dysfunction by stimulating extracellular matrix (ECM) protein deposition, hypertrophy, renal tubular damage, and increased water, glucose, albumin, and electrolyte secretion (Chang et al., 2016). However, suppressing TGF-β1 by monoclonal antibodies reduced diabetic renal complications, the renal uptake of glucose, and the excretion of albumin and water, thus suggesting a novel therapeutic target (Ziyadeh et al., 2000; Sha et al., 2017; Zhao et al., 2020).

Therefore, it seems reasonable that controlling hyperglycemia and/or attenuating oxidative stress and suppressing renal TGF-β1 could provide novel targets that protect against DN. In some areas of the world, including the Arabian Peninsula region, people regularly consume the garden cress (Lepidium sativum) herb (Shukla et al., 2012). Its traditional use is supported by its ability to alleviate cardiovascular and renal disorders, treat hypertension, and stimulate immune function and bone healing (Maier et al., 1998; Eddouks et al., 2002; Yadav et al., 2011; Attia et al., 2019). Several experimental studies have also revealed the hypoglycemic, hypolipidemic, and antioxidant protective effects of *L. sativum* in animal models. Indeed, in a recent study, the authors discovered that *L. sativum* significantly reduced blood glucose levels ameliorated the impairment in the lipid profile, and stimulated liver antioxidant enzyme levels in a T2DM-induced animal model fed a high-fat diet (Chen et al., 2020). Similar hypolipidemic and hypoglycemic effects with a potent systemic antioxidant effect of aqueous and methanolic aqueous *L. sativum* were also reported in alloxan-induced T1DM in rats (Shukla et al., 2012; Attia et al., 2019). It also attenuated hyperglycemia and prevented epididymis damage in diabetic rats brought on by streptozotocin (STZ) (Kamani et al., 2017).

Yet, the renal protective effect of *L. sativum* in a diabetic animal model has still not been established. Also, the renal protective mechanism is still unknown. Thus, our research provides the first evidence for the impact of *L. sativum* on nephron protection in rats with STZ-induced T1DM. In addition, we demonstrate that this protection offers potent hypoglycemic and antioxidant impacts as well as the downregulation of TGF-1β, which could be independent or interconnected effects.

2. Experimental design

2.1. Models (rats)

Male Wistar Albino rats (weighing 220 ± 15 gm) were obtained from the Experimental Animal Care Center at King Saud University, Riyadh, Saudi Arabia. The temperature and humidity of the housing were 22 ± 5 °C and 60%, respectively, with 12 h of light and 12 h of darkness. All rats adapted for 1 week and then were randomly selected for the experimental procedure described below. The King Saudi University's Research Ethics Committee (REC) in Riyadh, Saudi Arabia, gave its approval to all procedures used on these animals (No. KSU-SE-22–08).

2.2. Chemicals

Assay kits for the measurements of the fasting plasma glucose, serum cholesterol (CHOL), and tissue levels of malondialdehyde (MDA) were purchased from Cayman Chemicals, USA (Cat. I. D.10009582, Cat. I.D.10010303, and Cat. I.D. 10009055, respectively). ELISA kits to measure the fasting plasma insulin were provided by Ann Arbor, USA (Cat. I.D. 589501). The analysis kits of the serum/urine total creatinine (Cr)/albumin, NF-κB p65, and SOD were collected from MyBioSource, USA (Cat. I.D. MBS841754; Cat. I.D. MBS036924, and Cat. I.D. MBS2505513, respectively). Analysis of the serum urea levels was conducted using a commercial protocol kit from Bioassay Systems, USA (Cat. I.D. DIUR-100). The level of total glutathione was assayed by an assay kit from Biobit Chemicals, USA (Cat. ID. orb782371). TNF-α and IL-6 levels were measured by an ELISA kit provided by ThermoFisher, Germany, and R&D System, Minneapolis, MN, USA, respectively (Cat. ID. BMS622 and Cat. I.D. R6000B, respectively). A kit for total RNA extraction was purchased from Qiagen, Germany (Cat. I.D. 74004). The ThermoFisher kit (Cat. I.D. K1621) was employed for the construction of the first-strand cDNA. The Sofas Evergreen Supermix kit for cDNA amplification (Cat. I.D. 172–5200) was collected from BioRad, USA). The protocol kit Cat. I.D. ECCH-100 was used to analyze serum triglycerides (TGs) and was purchased from Bioassay Systems, USA. The protocol kit, Cat. I.D. 79960, for the measurement of low-density-lipoprotein-cholesterol (LDL-c) levels was supplied by Crystal Chemicals, USA (Cat. I.D. 79960).

2.3. Aqueous extraction of L. sativum

Fresh, whole-plant garden cress (*L. sativum*) was collected from a market in Riyadh, KSA, and identified by the Department of Plant Taxonomy and Pharmacognosy at the College of Pharmacy, KSA. The whole plant was washed with tap water, air-dried, and then extracted in a Soxhlet apparatus for 2 h (0.1 kg/1 L) (Berroukche et al., 2018). Then, using a rotatory evaporator, the excess water was dried, and the collected dried material was frozen. This dried material was then re-dissolved in normal saline at the desired concentration used for the experimental procedure.

2.4. Establishment of T1DM

T1DM was introduced by injection of 65 mg/kg STZ dissolved in Na-citrate as previously established in our labs (Altamimi et al.,2020). Diabetes became apparent in rats 72 h later when fasting blood glucose surpassed 320 mg/dL. In addition, random pancreatic sections were used to confirm β-cell damage (data not shown). A single dose of 0.5% glucose was given to every rat directly after the injection of STZ to avoid death from sudden hypoglycemia. All glucose measurements were performed using a commercial glucometer.

2.5. Experimental design

Rats with and without STZ-prompted T1DM were chosen at random and divided into (n = 8): (1) Non-diabetic control group: Normal healthy treated with 0.5 mL normal saline (vehicle); (2) *L. sativum*-treated control group: non-diabetic rats treated with 0.5 mL of *L. sativum* at a dose of 250 mg/kg; (3) STZ-T1DM model group: STZ-diabetic rats treated with 0.5 mL of normal saline; (4) STZ-T1DM + sativum-treated group: STZ-diabetic rats treated with 0.5 mL of *L. sativum* at a final dose of 250 mg/kg. The rats' body weight and food intake were recorded daily. The whole experimental period lasted 8 weeks.

2.6. Selection of L. sativum therapeutic dose

The dosage of *L. sativum* used in this study was adapted from previous research conducted by Shukla et al. (2012), who discovered that the plant's aqueous extract had a significant hypolipidemic and hypoglycemic impact on rats with alloxan-induced T1DM. In addition, the efficiency of this dose in lowering the fasting blood glucose levels in rats was tested in our labs using a pilot increasing-dose study (50–300 mg/kg), which showed similar potent effects at the highest doses (250 and 300 mg/kg).

2.7. Blood sampling and analysis

Experimental rats were transferred to metabolic cages and received food and water on day 56. Then, 24 h were spent collecting urine samples. Then, over the following 12 h, the rats were put back into their cages. Following this, ketamine (65 mg/kg) was used to anesthetize all rats. Blood samples were then collected into EDTA and plain tubes, and the serum and plasma were separated by centrifuging (3000 rpm; 10 min). Before use, all urine and blood samples were frozen at −20 °C, then thawed for analysis.

2.8. Collection of tissues and faces

Directly after the blood collection, the rats were ethically killed by cervical dislocation. Kidneys were then taken out, cleaned in ice-cold phosphate-buffered saline (PBS) (pH 7.4), freed from fat tissues, and weighed. The kidneys were split into sections of 1–2 mm^3 size. Some sections were maintained in 10% buffer formalin, while the rest were frozen at −20 °C until analysis. In the same way, the livers were likewise obtained and snap-frozen at −80 °C. Additionally, the stool of each rat was gathered during the final week of the animal trial, dried at 60 °C, and stocked in the fridge at 4 °C.

2.9. Biochemical analysis in the blood and stool

PBS (pH = 7.3) was used to homogenize part of the frozen kidney sections, followed by centrifugation at 10,000 rpm to separate the supernatant, which was then kept at − 80 °C. Frozen livers and stool lipids were separated following the method referred to by Folch et al. (1957). The amounts of albumin and Cr in the urine, urea, albumin, Cr, TGs, CHOL, FFAs, and LDL-c in the serum, and glucose and insulin in the plasma were determined using the aforementioned technique or ELISA kits. The amounts of lipids in the stool and liver were established using the same assay kits as in the serum. The MDA, GSH, SOD, TNF-α, NF-κB p65, and IL-6 levels were measured using their specialized kits. In compliance with the manufacturer's recommendations, all samples were examined using n = 8 samples per group.

2.10. Real-time PCR (qPCR)

TGF-β1, SOD, and catalase (CAT) mRNA levels were assessed using qPCR. Primers used for this part were supplied by ThermoFisher, USA. The primer sequence for TGF-β1 was adapted from Qu et al. (2009), whereas the primer sequences for SOD and CAT were adapted from Cappelli et al. (2018). In brief, the RNA and first-strand cDNA were prepared using commercial kits. Then, the qPCR was conducted in a Biorad qPCR amplification machine with the use of the Ssofast Evergreen Supermix kit and as per the manufacturer's instruction. The following settings were used for the amplification: heating (1 cycle/98 °C/30 s), denaturation (40 cycles/98 °C/5 s), annealing (40 cycles/60 °C/5 s), and melting (1 cycle/95 °C/5 s/step). The relative mRNA TGFβ-1 was shown after normalizing that of β-actin utilizing the $2\Delta\Delta^{CT}$ approach.

2.11. Histology study

Formalin-preserved tissue was deparaffinized in xylene, followed by sequential rehydration in different ethanol solutions of decreasing percentages (100%, 95%, and 70%). After this, tissue samples were immersed in wax to form a thin wax layer and then cut into slices (3–5 μM thickness) by a rotatory microtome. The slices were then stained with Harris hematoxylin /glacial acetic acid solution, followed by de-staining with HCl/70% ethanol solution (1:400 *v*/*v*). The samples were re-stained with eosin. Following their dehydration with ethanol and xylene, the tissue samples were placed on glass slides and covered with coverslips after adding mounting media. The next day, the slides were observed under a light microscope, and images were taken.

2.12. Statistical analysis

Analysis of the data was performed on the GraphPad Prism software (Version 8, USA). The 1-way ANOVA was used to test significant differences among groups. Tukey's post hoc test was used to separate means at a significant level of 0.05. Data were expressed as means ± standard deviation (SD).

3. Results

3.1. L. sativum attenuates fasting hyperglycemia and hypoinsulinemia in rats

The fasting glucose amount in the STZ-T1DM-induced group was significantly higher, while the insulin level was significantly lower when compared with the control group (Fig. 1A, B). On the contrary, the STZ-T1DM group treated with *L. sativum* revealed a significant reduction in the fasting glucose level and an increase in insulin level compared to the T1DM-induced group (Fig. 1A, B). In addition, but with no change in fasting glucose, insulin levels in the control group dropped significantly after receiving a dose of *L. sativum* (Fig. 1A and B).

3.2. L. sativum attenuates weight gain and hyperlipidemia in diabetic rats

In the control model rats, the body weight, serum levels of TGs, CHOL. LDL-c, FFAs, and hepatic TG, CHOL, and FFAs levels increased significantly after the STZ-induction of T1DM (Table 1). All of these parameters were significantly lower in the T1DM + *L. sativum*-treated group when compared with the T1DM group (Table 1). Compared to all other rat groups, the kidney weights were significantly lower in the T1DM group (Table 1). Interestingly, serum and hepatic lipid markers were significantly lower in the control

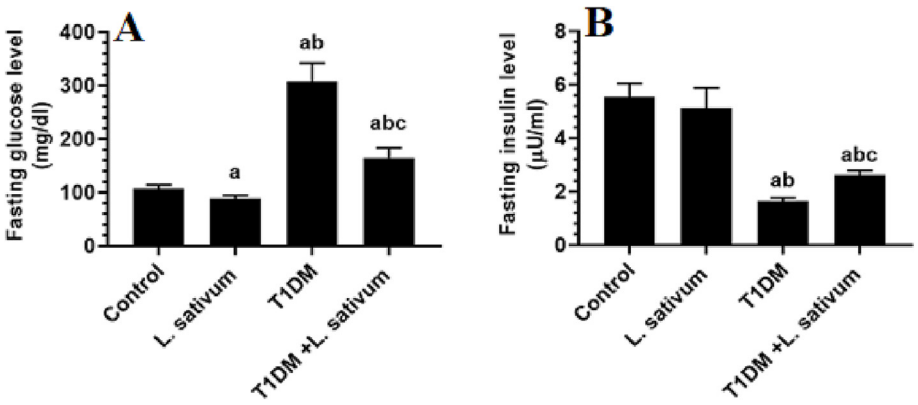

Fig. 1. Fasting plasma glucose and insulin levels in all groups of rats (means ± SD; n = 8 rats/groups). [a]: vs. control; [b]: vs. *L. sativum* (250 mg/kg)-treated rats; [c]: vs. STZ-induced T1DM rats; and [d]: vs. T1DM + *L. sativum* (250 mg/kg)-treated rats.

Table 1
Lipid marker profiles in all groups of rats.

		Control	*L. sativum*	T1DM	T1DM + *L. sativum*
Weights (g)	**Final body weight**	463 ± 31	453 ± 48	333 ± 28 [ab]	429 ± 31.5[c]
	Kidney weight	1.73 ± 0.17	1.81 ± 0.21	1.34 ± 0.18 [ab]	1.79 ± 0.18[c]
Serum	**TGs (mg/dL)**	74.9 ± 4.6	61.2 ± 5.8 [a]	132 ± 11.4 [ab]	85.4 ± 7.1 [bc]
	CHOL (mg/dL)	95.6 ± 8.4	79.5 ± 6.4 [a]	172 ± 13.9 [ab]	103 ± 11.9 [bc]
	LDL-c (mg/dL)	46.5 ± 7.1	33.5 ± 4.3 [a]	92.3 ± 8.4 [ab]	58.7 ± 6.8 [abc]
	FFAs (µmol/L)	784 ± 66.9	619 ± 44.9 [a]	1240 ± 113 [ab]	983 ± 88.4 [abc]
Liver	**TGs (mg/g)**	5.9 ± 0.54	4.4 ± 0.39 [a]	9.9 ± 1.7 [ab]	6.4 ± 0.71 [abc]
	CHOL (mg/g)	4.1 ± 0.58	3.3 ± 0.48 [a]	8.9 ± 0.54 [ab]	5.6 ± 0.64 [abc]
	FFAs (µmol/g)	148 ± 15.4	119 ± 14.3 [a]	431 ± 29.4 [ab]	221 ± 14.6 [abc]

Data are presented as means ± SD (n = 8/group). [a]: vs. control; [b]: vs. *L. sativum* (250 mg/kg)-treated rats; [c]: vs. STZ-induced T1DM rats; and [d]: vs. T1DM + *L. sativum* (250 mg/kg)-treated rats.

rats given *L. sativum* than the untreated control rats, despite no difference in final body weight (Table 1).

3.3. *L. sativum restores kidney function marker levels in T1DM-treated rats*

No significant variations existed between the control and *L. sativum*-treated control in the results of any kidney function tests (Table 2). In T1DM-treated rats compared to control rats, the urine volume, albumin, serum urea, and Cr levels were significantly increased, whereas the urine Cr levels significantly decreased (Table 2). When compared to T1DM model rats, the markers in the T1DM + *L. sativum*-treated rats reverted to their basal levels (Table 2).

3.4. *L. sativum attenuates renal inflammatory makers and oxidative stress and suppresses the expression of TGF-β1*

The mRNA concentrations of SOD and CAT and the amounts of SOD, GSH, and CAT, were significantly higher in the control rats administered *L. sativum* as compared with the control rat alone, while the TGF-β1 expression was significantly lesser (Fig. 2A–C and 3A–C). No significant differences in TNF-α, IL-6, and NF-κB levels in the kidneys were seen between the control and the rats treated with *L. sativum* (Fig. 4A–C). In comparison with the control group, the T1DM-treated group displayed significantly spiked expression levels of the total SOD, GSH, and CAT and the mRNA SOD and CAT, as well as significantly declined expression levels of the total TNF-α, IL-6, and NF-κB and the mRNA TGF-β1 (Fig. 2A–C, 3A–C, and 4A–C). When compared to T1DM model rats, these biochemical markers were markedly reversed in the T1DM rats treated with *L. sativum* (Fig. 2A–C, 3A–C, and 4A–C).Fig. 3.

3.5. *L. sativum preserves pancreas and liver histology*

Fig. 5A,B, and Fig. 6A,B showed that the control and *L. sativum*-treated rat groups had normal pancreas structures with intact β-cells, α-cells, and islets of Langerhans, as well as normal kidney structures with perfect proximal convoluted tubules (PCTs), distal convoluted tubules (DCTs), glomerulus, and glomerular membrane. On the other hand, shrinkage in the size of the islets, reduced numbers of α- and β-cells, and severe damage to the DCTs

Table 2
Kidney function markers in all groups of rats.

		Control	*L. sativum*	T1DM	T1DM + *L. sativum*
Serum	**Urea (mg/dL)**	7.8 ± 1.4	8.3 ± 2.1	36.4 ± 3.9 [ab]	14.6 ± 2.8 [abc]
	Creatinine (mg/dL)	0.73 ± 0.13	0.69 ± 0.11	1.9 ± 0.15 [ab]	0.96 ± 0.11 [abc]
Urine	**Volume (mL)**	12.3 ± 2.3	11.8 ± 2.1	19.3 ± 2.9 [ab]	12.2 ± 1.9 [bc]
	Albumin (µg/dL)	22.3 ± 2.9	20.9 ± 3.1	67.4 ± 5.9 [ab]	33.2 ± 3.1 [abc]
	Creatinine (mg/dL)	93.4 ± 8.1	88.3 ± 7.9	22.2 ± 2.9 [ab]	76.3 ± 6.5 [abc]

Data are presented as means ± SD (n = 8/group). [a]: vs. LFD, [b]: LFD + apigenin-treated rats, [c]: Data are presented as means ± SD (n = 8/group). [a]: vs. control; [b]: vs. *L. sativum* (250 mg/kg)-treated rats; [c]: vs. STZ-induced T1DM rats; and [d]: vs. T1DM + *L. sativum* (250 mg/kg)-treated rats.

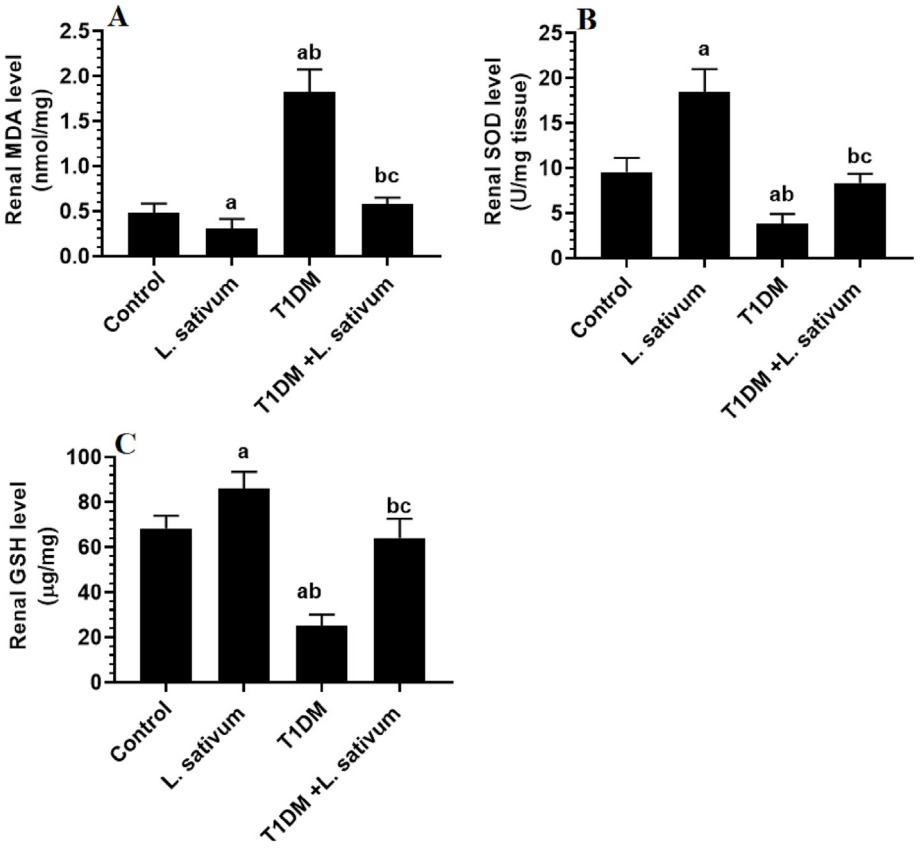

Fig. 2. Levels of markers of oxidative stress/antioxidants (A, B, and C) in the kidneys of all groups of rats. Data are presented as means ± SD (n = 8/group). [a]: vs. control; [b]: vs. *L. sativum* (250 mg/kg)-treated rats; [c]: vs. STZ-induced T1DM rats; and [d]: vs. T1DM + *L. sativum* (250 mg/kg)-treated rats.

and PCTs were seen in the T1DM model rat's kidneys (Fig. 5C and Fig. 6C). Furthermore, the T1DM + *L. sativum*-treated group showed almost normal PCTs and DCTs, and the size of the islets of the Langerhans and the number of β-cells were significantly increased (Fig. 5D and Fig. 6D).

4. Discussion

In our study, STZ-induced T1DM resulted in hyperglycemia, hyperinsulinemia, severe renal damage associated with microalbuminuria and oxidative stress, and kidney inflammation. Several observations, including an upsurge in the generation of MDA, NF-κB, TNF-α, and IL-6 and a concomitant reduction in amounts or expressions of GSH, SOD, and CAT proved this assertion. In addition, STZ-T1DM led to a significant elevation of TGF-α, an essential player protein that could regulate numerous functions related to kidney health, including the fibrosis process, oxidative stress, glucose uptake, inflammation, and the excretion of ions. Further, the STZ-diabetic rats receiving a dose of the *L. sativum* extract attenuated the observed nephropathy and reversed all the events mentioned above. Treating the control rat with *L. sativum* also significantly lowered the fasting glucose and renal TGF-β1 levels, while boosting the SOD and CAT in the kidneys. These findings imply that the nephroprotection of *L. sativum* is mediated by its hypoglycemic and antioxidant effects and inhibits the activation of TGF-β1.

STZ is the preferred drug used in experimental rodents to cause T1DM, which is administered at 55–65 mg/kg (Wang-Fischer and Garyantes, 2018; Aldayel et al.,2020). The diabetogenic role of STZ is mediated by the specific induction of pancreatic oxidative stress through activating ROS-generating enzymes and elevating the levels of free radicals and ROS (Wang-Fischer and Garyantes, 2018). At this dose of STZ, several studies have shown that 60–80% of the pancreatic β-cells are damaged, thus leaving about 20–40% of active cells which can produce insulin (Wang-Fischer and Garyantes, 2018). This results in rapid severe hypoinsulinemia and hyperglycemia, which over time, promotes oxidative and inflammatory damage to organs, and diabetic health problems eventually arise (Aldayel et al.,2020). Moreover, despite the increase in appetite, the treatment of STZ is linked to a loss in body weight, resulting from muscle wasting and a depletion of glycogen contents in the liver and muscle in response to impaired insulin availability and action (Montano et al., 2010; Aldayel et al.,2020). In agreement with these studies, the histological findings of this study have revealed the selective destruction of β-cells of STZ-prompted diabetic rats, which also showed severe hyperglycemia, hypoinsulinemia, and reduced body weights throughout the study.

In both the control and STZ-prompted diabetic groups, *L. sativum* significantly lessened the fasting glucose levels and increased the level of circulatory insulin. It also restored the normal size of the islet of the Langerhans of the β-cells in the STZ-diabetic rats. The considerable increase in rat body weight may be explained by the elevated insulin level in the plasma. These results reveal that *L. sativum's* hypoglycemic impact is partially mediated by reducing the β-cell ongoing damage and regeneration, which could be due to this plant's antioxidant and anti-inflammatory effect, as discussed below. These results conformed to those reported by Chen et al. (2019), who also reported a generating potential of *L. sativum* on the STZ/HFD-diabetic rat β-cells. Additionally, treatment with *L. sativum* increased insulin levels and reduced fasting hyperglycemia in diabetic rats (Attia et al., 2019). Another possible explanation for *L. sativum's* hypoglycemic effect could be its

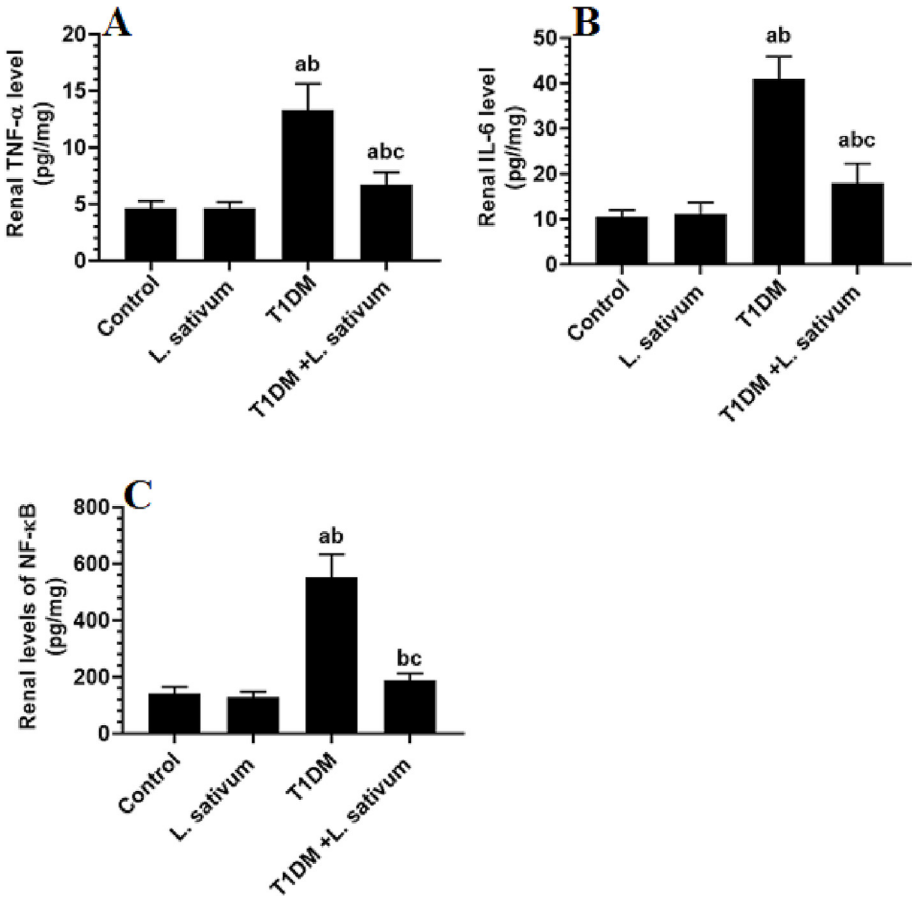

Fig. 3. Levels of markers of inflammation in the kidneys of all groups of rats. Data are presented as means ± SD (n = 8/group). [a]: vs. control; [b]: vs. *L. sativum* (250 mg/kg)-treated rats; [c]: vs. STZ-induced T1DM rats; and [d]: vs. T1DM + *L. sativum* (250 mg/kg)-treated rats.

enhancement of peripheral insulin action and modulation of hepatic glucose homeostasis.

The most common way to identify kidney-related disorders is to evaluate key kidney markers and the histological studies of renal biopsies (Severin et al., 2019, Pourghasem et al., 2015). Higher blood Cr and albuminuria levels are major clinical features in the majority of renal disorders, including DN (Severin et al., 2019, Pourghasem et al., 2015). In addition, DN is described by renal microvasculature disruption, glomerular thickening, and renal tubule injury (Kashihara et al., 2015). In the same line of this evidence, we have also confirmed DN in STZ-diabetic rats by the apparent damage in the glomerular and renal tubules, and by the dysfunctional markers of the kidney function, the significant increase in the plasma urea and Cr, and the increase in the urine volume and albumin levels. The *L. sativum's* ability to attenuate all these abnormalities was our first evidence of its nephroprotective effect. Since hyperglycemia is the major contributing factor of DN, our findings strongly support that the nephroprotective effect of *L. sativum* could be attributed to its hypoglycemic and insulin-releasing activities.

On the other hand, hyperglycemia can induce DN by promoting oxidative stress and inflammation, which have been described as the two major upstream damaging pathways (Mima, 2013). Inflammation and oxidative stress are interrelated pathways that act in vicious positive feedback mechanisms to regulate each other (Mima, 2013) positively. In response to hyperglycemia, high levels of ROS are generated due to the activation of numerous enzymatic and non-enzymatic pathways and the impaired oxidative phosphorylation of the mitochondria (Mahmoodnia et al., 2017). These ROS can damage cell membranes and organelles by inducing lipid peroxidation (Mahmoodnia et al., 2017). In addition, ROS can damage the cells by depleting enzymatic and non-enzymatic antioxidants (Liu et al., 2017). Furthermore, ROS triggers inflammation by activating the NF-κB transcription factor, which subsequently induces the production IL-6 and TNF-α (Malik et al., 2017; Wang et al., 2017). These inflammatory cytokines can impair insulin signaling and exaggerate oxidative stress by producing more ROS (Wang et al., 2017). In addition, ROS and inflammatory cytokines can damage renal tissue by inducing apoptosis and fibrosis (Wang et al., 2017). Interestingly, and without modulating glucose levels, the administration of anti-inflammatory and antioxidant medicines and the upregulation of antioxidants or the deficiency of TNF-α protected against hyperglycemia-induced DN (Mima,2013; Ali et al., 2017; Malik et al., 2017; Eleazu et al., 2022).

In this investigation, our data show that *L. sativum* also exhibited significant anti-inflammatory and antioxidant capabilities in addition to the nephroprotection that was seen. This is proved by the drop in levels of lipid peroxides generated (MDA) in rats with diabetes caused by STZ, as well as the NF-κB, IL-6, and TNF-α in their kidneys. In addition, the *L. sativum* treatment significantly raised levels of total and mRNA renal antioxidant enzymes CAT and SOD in these diabetic rats, as well as total renal GSH. Although these effects could be a result of the parallel hypoglycemic effect of *L. sativum*, which is expected to reverse these damaging pathways, an interesting observation seen here was the ability of *L. sativum* to act positively on the GHS, SOD, and CAT in control group too, thus confirming that it has independent antioxidant activities. In addition, *L. sativum's* stimulation of antioxidant markers in the kidneys

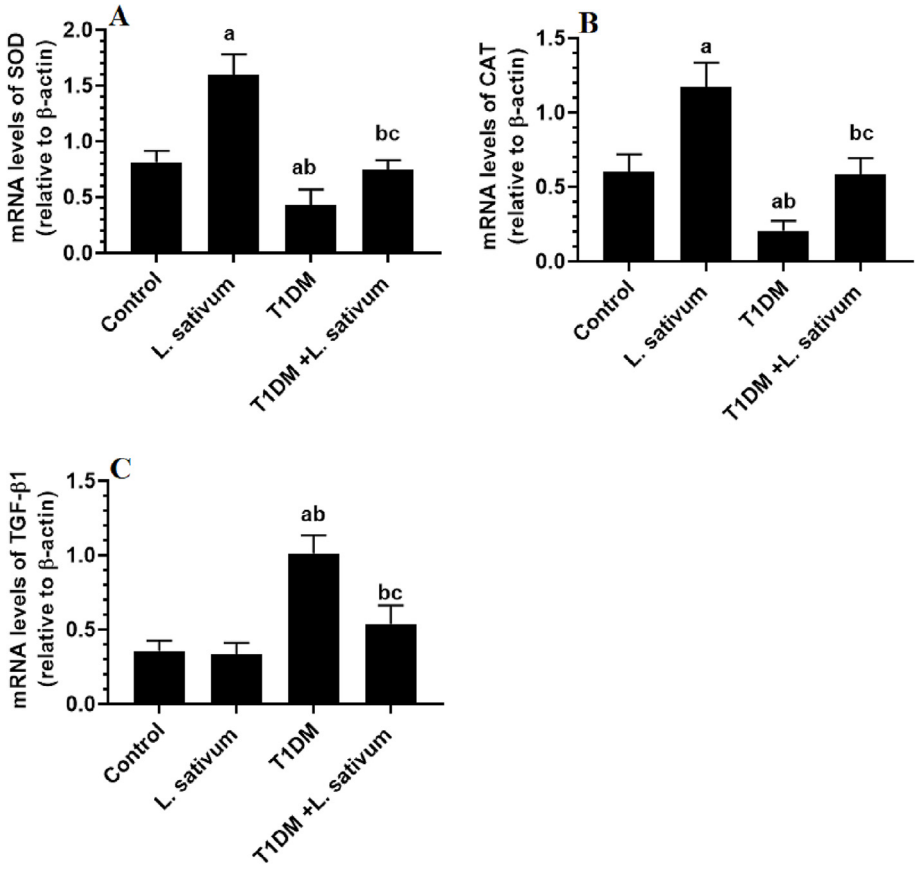

Fig. 4. mRNA levels of selected markers in the kidneys of all groups of rats. Data are presented as means ± SD (n = 8/group). [a]: vs. control; [b]: vs. *L. sativum* (250 mg/kg)-treated rats; [c]: vs. STZ-induced T1DM rats; and [d]: vs. T1DM + *L. sativum* (250 mg/kg)-treated rats.

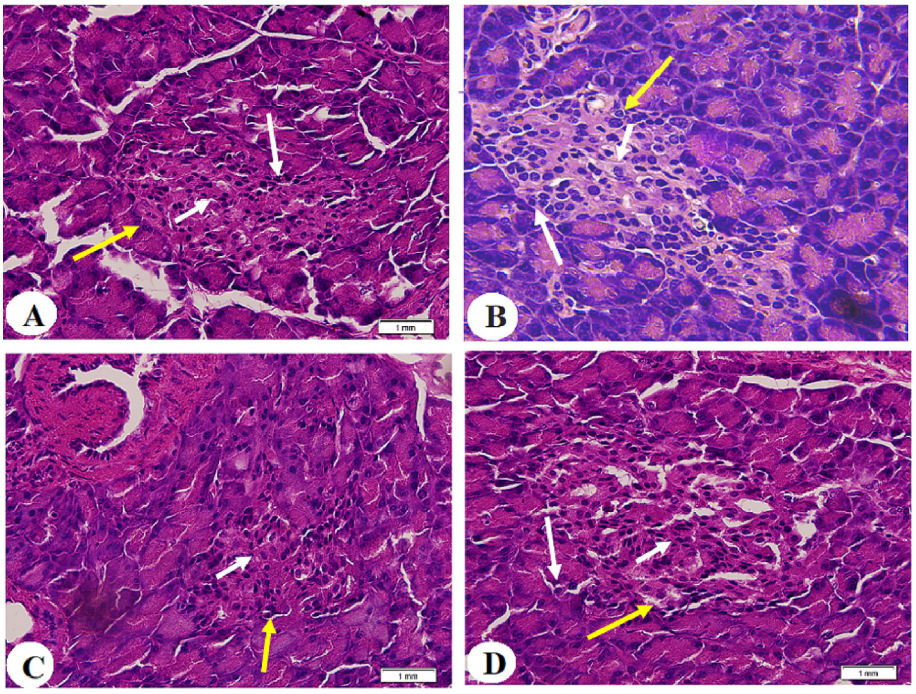

Fig. 5. Histological images of the pancreases of all groups of rats stained with H&E. 200X. (A and B) Taken from control and *L. sativum*-treated rats, showing normally sized islets (yellow arrows) with intact α and β-cells (long and short white arrows, respectively. (C) Taken from T1DM-treated rats showing obvious shrinkage in the size of the islets (yellow arrow) with a reduced mass of β-cells (short white arrow) and signs of edema. (D): Taken from T1DM + *L. sativum* rats, showing increased islet size (yellow arrow) and a number of β-cells (short white arrow).

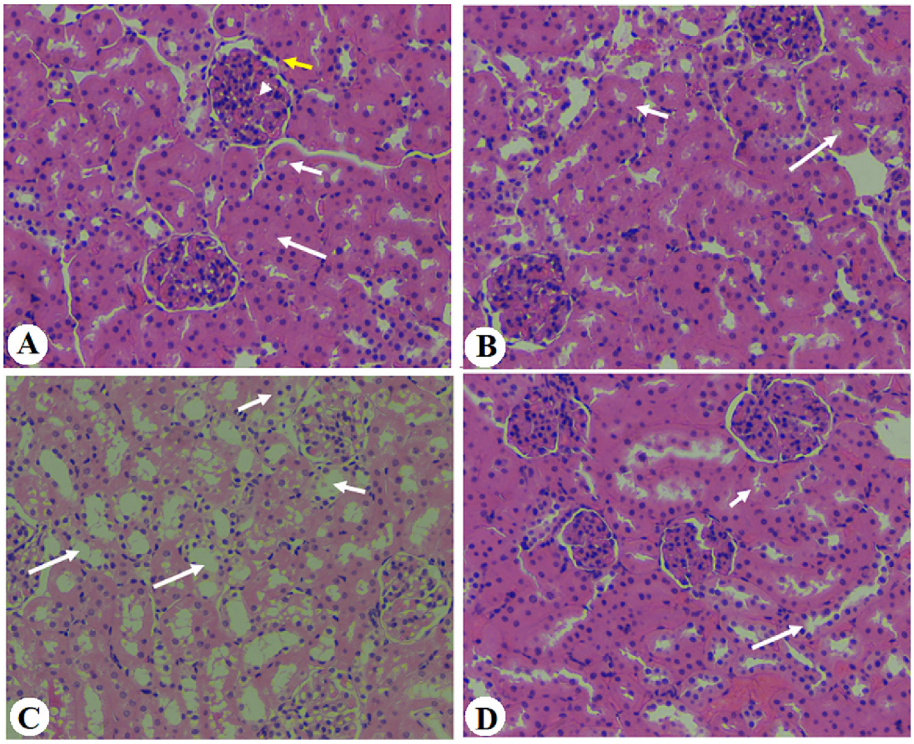

Fig. 6. Histological images of the kidneys of all groups of rats as stained with H&E. 200X. (A and B) Taken from control and *L. sativum*-treated rats, showing normal glomerulus (arrowhead), glomerular membrane (short yellow arrow), proximal convolutes tubules (PCTs) (short white arrow) and distal convolutes tubules (DCTs) (long white arrow). (C) Taken from T1DM-treated rats, showing severe damage in the PCTs (short white arrow) and DCTs (long short arrow). (D) Taken from T1DM + *L. sativum*, showing much improvement in the structures of PCTs and DCTs. However, some damage can still be seen, mainly in the PCTs.

of the control, non-diabetic rats suggests that its antioxidant potential is an upstream mechanism that regulates its anti-inflammatory effect observed in the diabetic rat kidneys. This backs up previous studies, showing oxidative stress is the major mechanism in diabetic kidney damage that activate all other deleterious pathways, such as inflammation, apoptosis, and fibrosis (Malik et al., 2017; Wang et al.,2017; Eleazu et al., 2022). These findings support the results obtained by Chen et al. (2020), who also showed that treatment with *L. sativum* can prevent liver damage in diabetic rats by suppressing lipid peroxidation, increasing GSH content, and raising glutathione peroxidase (GPx), CAT, and SOD levels.

Hyperlipidemia is a major manifestation associated with T1DM in humans and STZ-treated animals and is a key risk factor for DN by promoting lipotoxicity and mitochondria damage (Montano et al., 2010; Almeida et al., 2012; Okoduwa et al., 2017; Chen et al., 2021). In this context, the deficiency of circulatory insulin inhibits the peripheral lipoprotein lipase hormone, which normally stimulates adipose tissue lipogenesis and fat synthesis (Aldayel et al.,2020). However, this causes the liver to receive an increased amount of free fatty acids, which eventually stimulates the creation of TGs and cholesterol (in particular LDL-c), thus resulting in dyslipidemia (Mourrieras et al., 1997; Aldayel et al.,2020). Additionally, hyperglycemia alone is an independent factor that stimulates CHOL and TGs creation in the liver by upregulating the sterol regulatory element-binding transcription factors (SREB1/2) and their downstream fat synthesis genes (Matsuzaka et al., 2004; Mohamed et al., 2019). Previous investigates have shown higher levels of TGs, FFAs, LDL-c, VLDL-c, and CHOL, a significant decrease in the serum and liver levels, and increased TG and CHOL deposition in the STZ-diabetic rat kidneys (Almeida et al., 2012; Okoduwa et al., 2017; Chen et al., 2019; Aldayel et al.,2020). The findings of this study confirm that hyperlipidemia is a major contributing factor in T1DM-induced microvascular complications, including DN. On the contrary, these results imply that the nephroprotective capabilities of *L. sativum* can be attributed to its hypolipidemic effect and its capacity to attenuate renal lipotoxicity. Indeed, treatment with *L. sativum* significantly restored normal serum and hepatic levels of CHOL, LDL-c, and TGs, as well as FFAs levels in the serum and liver were significantly repressed. Therefore, it could be possible that such a hypolipidemic effect of *L. sativum* is attributed to independent or cooperating mechanisms mediated by acting on hepatic lipid synthesis geneses and the suppression of adipose tissue lipolysis by stimulating insulin levels/action. Supporting our data, the hypolipidemic effect of the administration of *L. sativum* has also been previously established in a variety of animal models, including those of T1DM, induced by alloxan, and T2DM, induced by HFD. Yet, further reports examining the effect of this plant on SREBPs will provide further insights into the precise mechanism underlying this effect and are highly encouraged (Ding et al., 2010; Attia et al., 2019, Chen et al., 2019; Salama et al., 2019).

However, despite controlling the blood sugar and the adoption of anti-inflammatory and antioxidant agents and other medications such as renin–angiotensin–aldosterone system (RAAS) inhibitors and sodium-glucose co-transporter 2 (SGLT2), the DN and chronic kidney disease treatment needs are still unmet (Zhao et al., 2020). Therefore, authors have suggested that targeting abnormal signaling pathways may help us better understand the pathology of DN and find a suitable and effective cure. TGF-β1 is a pleiotropic cytokine created by all kidney cells and infiltrating macrophages. It is involved in regulating numerous cell processes, including immunomodulation, angiogenesis, wound healing, and the synthesis of ECM. TGF-β1 cells are abnormally spiked in the diabetic rat kidney, as well as other renal disorders at all phases of the disease (Schena and Gesualdo, 2005; Zhao et al., 2020).

Reports have shown the serious adverse effect of this activation, including induction promoting tubular and glomerular degeneration by activating leukocyte infiltration, inflammation, oxidative stress, autophagy, and fibrosis (Inoki et al., 1999; Meng et al., 2010; Zhao et al., 2020). Besides, TGF-β1 stimulates the renal cell uptake of glucose by overexpressing the GLUT-1 receptors, which accelerates the glucose metabolism in these cells (Inoki et al., 1999; Schena and Gesualdo, 2005). Interestingly, pharmacological inhibition, blockage by neutralizing antibodies, and the downregulation of TGF-β1 alleviated kidney structural and functional changes in experimental animals with T1DM and T2DM (Zhao et al., 2020). Therefore, it has been suggested that modulating TGF-β1 could be a novel tactic to treat DN.

In conclusion, based on our data, we suggest *L. sativum* as a potential therapy to alleviate DN due to its antioxidant, hypoglycemic, and TGF-β1 suppressing capabilities. However, more validation of these conclusions will require experiments. Future subclinical trials will also be necessary to confirm this effect in people.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was funded by Researchers Supporting Project, King Saud University, Riyadh, Saudi Arabia, grant number RSPD2023R638.

Acknowledgments

The authors extend thanks to the Researchers Supporting Project number (RSPD2023R638), King Saud University, Riyadh, Saudi Arabia.

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