$(\mathbf{i})$ 

#### THIEME OPEN ACCESS

# Nephroprotective Potential of Sphaeranthus indicus Linn Extract against Hyperglycemia and Dyslipidemia in Streptozotocin-Induced Diabetic Nephropathy

Vishal B. Jadhav<sup>10</sup> Jai Singh Vaghela<sup>10</sup>

<sup>1</sup> Department of Pharmacology, Bhupal Nobles' College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India

J Health Allied Sci<sup>NU</sup> 2024;14:210–218.

Address for correspondence Vishal B. Jadhav, PhD, Department of Pharmacology, Bhupal Nobles' College of Pharmacy, Bhupal Nobles' University, Udaipur-313001, Rajasthan, India (e-mail: kavyashri9312@yahoo.com).

# Abstract

**Objective** This study was aimed at determining the nephroprotective potential of *Sphaeranthus indicus* Linn methanol extract (SME) against hyperglycemia and dyslipidemia in streptozotocin (STZ)-induced diabetic nephropathy (DNP) in adult Wistar albino rats.

**Materials and Methods** Following STZ-induced diabetes, adult albino Wistar rats of either sex with serum glucose level more than 250 mg/dL were chosen and randomized into six groups (n = 6 rats per group) and received the treatment as follows: Group I: Normal nondiabetic (ND) rats received a single intraperitoneal dose of citrate buffer in the same volume as STZ and 1% (w/v) carboxymethyl cellulose (CMC) per os (po), group II: diabetic (STZ) control rats received oral dosage of 1% (w/v) CMC, group III, IV and V: STZ + SME treated rats received a suspension of SME (100, 200, and 400 mg/kg, po) in 1% (w/v) CMC, and group VI: STZ + MET treated rats received metformin (500 mg/kg, po) as suspension in 1% (w/v) CMC. From 28th day to the 56th day of STZ injection, SME and MET were given for 28 days in the form of freshly prepared suspension. The impact of STZ-induced DNP was analyzed through the estimation of body weight, serum glucose, and hemoglobin A1c levels, renal functional parameters, the serum lipid profile, oxidative stress markers, and analysis of renal histoarchitecture.

# Keywords

- ► streptozotocin
- diabetic nephropathy
- Sphaeranthus indicus
- hypoglycemic
- antioxidant
- hypolipidemic

serum glucose and hemoglobin A1c levels, renal functional parameters, the serum lipid profile, oxidative stress markers, and renal histoarchitecture in contrast to normal ND rats. SME and MET treatment significantly reduced hyperglycemia-induced enhanced lipid profile and oxidative stress, normalized renal functional parameters, and restored renal histoarchitecture by reducing vacuolar degeneration of renal tubules in contrast to diabetic (STZ) control rats. These findings were attributed to SME's efficacy in DNP. **Conclusion** In STZ-sensitized diabetic rats, SME retarded the progress of nephropathy. The observed nephroprotective potential of SME is ascribed to its hypoglycemic, hypolipidemic, and antioxidant activities.

**Result** Diabetic (STZ) control rats showed significant alterations in body weight,

article published online June 13, 2023 DOI https://doi.org/ 10.1055/s-0043-1769511. ISSN 2582-4287. © 2023. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited. (https://creativecommons.org/licenses/by/4.0/) Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

# Introduction

A persistent metabolic illness, diabetes mellitus (DM), develops as a result of either damaged pancreatic  $\beta$  cells (insulin deficiency) or impaired absorption of glucose into cells (dysregulated insulin signaling).<sup>1,2</sup> It is widely recognized as multifactorial. The long-term microvascular and macrovascular consequences of diabetes, such as retinopathy, neuropathy, and nephropathy, are frequent.<sup>3</sup> Diabetic nephropathy (DNP) is a universal microvascular complication in diabetic individuals, illustrated by proteinuria, which leads to renal disorders.<sup>2,4</sup> It is believed that around 30% of diabetics worldwide develop DNP. Renal injury, oxidantperoxide stress, an inflammation, and fibrosis are the hallmarks of DNP developed due to altered glomerular hemodynamics. By vasodilating the afferent arteriole, chronic hyperglycemia leads to glomerular hyperfiltration by raising the hydrostatic pressure and increasing the flow of fluid through the glomerulus. Microalbuminuria, which is a sign of glomerular dysfunction, is brought on by modifications to the renal structure such as thickening of the basal membrane, podocyte lesions, and mesangial matrix expansion. These modifications eventually lead to glomerular sclerosis and tubulointerstitial fibrosis, both of which are linked to lower rate of filtration through glomeruli.<sup>5,6</sup>

Diabetes complications develop as a result of reactive oxygen species (ROS) generation, primarily superoxide anion (O<sub>2</sub><sup>-</sup>), causing cell malfunction and oxidative lesions via protein denaturation, lipid peroxidation, and mitochondrial DNA damage.<sup>7,8</sup> As a result of these alterations, nephrocytes such as glomeruloendothelial cells, mesangial cells, and renal epithelial cells have altered ATP production, intracellular calcium imbalance, and cell membrane permeability, all of which promote programmed and localized cell death.<sup>7,9</sup> The unifying element connecting abnormal renal hemodynamics with dysregulated metabolic pathways is chronic hyperglycemia-mediated unneeded ROS production.<sup>10,11</sup> Additionally, in the development of DNP, the deposition of endproducts of advanced glycation plays a crucial role.<sup>11,12</sup> Moreover, it has been shown that diabetic people have dyslipidemia and altered renal functional parameters.<sup>2,13,14</sup>

Despite having a mild-to-moderate side effect profile, existing antidiabetic medications provide good glycemic control but have a limited efficacy in controlling diabetes complications.<sup>15</sup> Though drugs acting on the renin-angiotensin system, like dipeptidyl carboxypeptidase I inhibitors and angiotensin II receptor blockers, are unable to delay DNP progression, they are reported to be valuable for progressive DNP.<sup>16</sup>

Diabetic patients have been treated with a variety of herbal medicine extracts in India since time immemorial. A variety of plants with antidiabetic qualities have been listed in Ayurveda. The herb *Sphaeranthus indicus* Linn has long been used in Indian tradition to cure several illnesses. It has several noteworthy biological properties including antioxidant, anti-inflammatory, antihyperglycemic, antihyperlipidemic, reno-protective, and other unspecified properties.<sup>17,18</sup>

The Sphaeranthus indicus Linn was examined for its nephroprotective potential against nondiabetic (ND) models of nephropathy such as gentamicin<sup>19,20</sup> and cisplatin<sup>21</sup> induced nephrotoxicity. An elevated levels of urea, creatinine, lipid peroxidation, and declined levels of total protein, albumin, superoxide dismutase, catalase, glutathione peroxidase activities, and reduced glutathione were significantly restored by ethanolic extract of *Sphaeranthus indicus* Linn along with reversal of features of acute tubular necrosis in nephrotoxic rats and restoration of renal histoarchitecture to normal indicating its renoprotective and antioxidant potential in animal models of non-DNP. Therefore, keeping in view the biological potentials, this work was undertaken to evaluate the effects of *Sphaeranthus indicus* Linn methanol extract (SME) in attenuating DNP in streptozotocin (STZ)-sensitized Wistar albino rats.

## Materials and Methods

#### Plant Collection, Authentication, and Extraction

An entire herb, *Sphaeranthus indicus* Linn collected at Pahine, Trimbakeshwar, indigenous to Nashik in August to September 2021, was certified by the Post-graduate Department of Botany at GES's HPT Arts and RYK Science Institution, Nashik, Maharashtra, India. The specimen sample (voucher number HPTRYK/342/2021–22) has been placed for future use. The dried apical parts, such as leaves, stems, fruits, and flowers, were coarsely powdered in a laboratory using a pulverizer. Soxhlet's extractor was used to prepare methanol extract of the powder. Extract concentration was done using a rotary evaporator at decreased pressure. The freeze-dried *Sphaeranthus* methanolic extract (SME) yield was calculated. SME was then analyzed primarily for the presence of bioactive constituents.<sup>22</sup>

## Chemicals

The USA's Sigma-Aldrich, located in St. Louis, supplied STZ (Product No. S0130) and metformin (Product No. 317240). All other analytically graded chemicals, reagents, and biochemical kits were bought from local suppliers. The reagents involved in the antioxidant assays were prepared in the laboratory before estimation.

#### Animals

Adult Swiss albino mice (6–8 weeks old, 20–25 g) and Wistar albino rats (150–200 g) of either sex were kept in conventional conditions of temperature ( $25^{\circ}C \pm 1^{\circ}C$ ), relative humidity (45–55%), a circadian cycle, and unrestricted food pellets and filtered water access. Before implementing the experimental technique, an acclimatization period (7–10 days) was followed. The ethical committee of the B. N. College of Pharmacy (870/PO/Re/S/05/CPCSEA) approved the experimental protocol (Proposal No. 24/BNCP/IAEC/2021), which was conducted in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guide-lines for animal testing.<sup>23</sup>

#### Experimental Methodology

#### Acute Oral Toxicity Study

SME's acute oral toxicity (AOT) was studied in accordance with Organization for Economic Cooperation and Development

(OECD) Guideline 425 to identify the median lethal dose ( $LD_{50}$ ) and to select the doses for further study.<sup>24</sup> Different oral doses of SME (1.75, 5.5, 17.5, 55, 175, 550, 1750, and 2000 mg/kg) suspended in 1% weight by volume (w/v) carboxymethyl cellulose (CMC) were administered to mice.<sup>25</sup> For up to 72 hours, the presence or absence of signs of toxicity or mortality was then observed in mice.

#### **Diabetes Induction and Assessment**

Diabetes was induced by injecting a single intraperitoneal (ip) dose of STZ (55 mg/kg, ip) diluted in citrate buffer (pH 4.4, 0.1 M). Forty-eight hours after STZ administration, blood was collected in Eppendorf tubes via the puncture of the retroorbital plexus. Measurement of serum glucose levels was done colorimetrically using the glucose oxidase-peroxidase (GOD-POD) kit from Accurex, India, after cold centrifugation of blood samples at 2,500 rpm for 15 minutes at 4°C by the GOD-POD method. Rats with serum glucose values greater than 250 mg/dL were confirmed as diabetics and further considered for this study.<sup>26</sup>

#### **Experimental Groups and Treatment**

Following the AOT study, the selected effective doses of SME were titrated against STZ-induced DNP. Adult albino Wistar rats were randomized into six groups (n = 6 rats per group) and received the treatment as follows: group I—normal ND received a single ip dose of citrate buffer in the same volume as STZ and an oral dose of 1% (w/v) CMC, group II—diabetic (STZ) control received oral dosage of 1% (w/v) CMC, groups III, IV, and V—STZ + SME received a suspension of SME (100, 200, and 400 mg/kg, per os [po] respectively) in 1% (w/v) CMC, and group VI—STZ + MET (500) treated rats received metformin (500 mg/kg, po) as suspension in 1% (w/v) CMC. From the 28th day to the 56th day of STZ injection, SME's effective doses (100, 200, and 400 mg/kg, po) and MET were given for 4 weeks in the form of freshly prepared suspension.<sup>26</sup>

#### **Assessment of Diabetic Nephropathy**

# Estimation of Body Weight, Serum Glucose, and Hemoglobin A1c Levels

At the conclusion of the 56th day, an alteration in the body weight (g) of rats was measured.<sup>27</sup> Blood was collected via tail venepuncture and placed in Eppendorf tubes containing disodium edetate. Following the cold centrifugation at 3,000 rpm for 15 minutes at 6°C, serum was separated and frozen at -20°C for later examination. A biochemical analyzer was used to measure glucose and hemoglobin A1c levels in half of the separated serum using the GOD-POD kit from Accurex, India, and the hemoglobin A1c (glycated) kit from Sigma Aldrich, USA, respectively, at the end of the 56th day.<sup>28</sup>

#### **Biochemical Estimation**

The remainder of the unused serum was analyzed for biochemical estimation of renal functional parameters like creatinine, blood urea nitrogen (BUN), uric acid, and the serum lipid profile of triglycerides (TG), total cholesterol (TC), and low, high, and very low-density lipoproteins (LDL-C, HDL-C, and VLDL-C) as well, using biochemical kits purchased from Arkray Healthcare Pvt. Ltd., Mumbai, India, at the end of the 56th day.

#### **Estimation of Oxidative Stress Markers**

At the end of the 56th day, under deep diethyl ether anesthesia, rats were sacrificed for the surgical removal of the kidneys. In 0.1 M ice-cold phosphate buffered saline with pH 7.4, the left kidneys were homogenized using a probe homogenizer (Polytron PT 2500E, Kinematica AG, Werkstrasse 7, c-d, 6102 Malters, Switzerland) and frozen at -20°C for assays of neural malondialdehyde (MDA),<sup>29</sup> reduced glutathione (GSH),<sup>30</sup> and superoxide dismutase (SOD).<sup>31</sup> The extent of MDA produced, the marker of peroxidation of lipid (LPO), was quantified by measuring thiobarbiturate acid-reactive substances present in 10% of the supernatant of neural homogenate.

#### Analysis of Renal Histoarchitecture

The right-side kidneys were promptly preserved in 10% formaldehyde and embedded in paraffin. The 5µm thick slices of renal tissues were cut using a microtome (Leica Biosystems, Germany). To evaluate the histoarchitectural alterations, certain tissue sections were deparaffinized and stained with hematoxylin and eosin. The signs of glomerular congestion, interstitial inflammatory infiltration, and atrophy of the glomerulus with dilated glomerular space were examined under 400x light microscopy.

#### **Statistical Analysis**

The datasets were presented as mean values ( $\pm$ standard error of mean) and analyzed by one-way analysis of variance trailed by *posthoc* Tukey' multiple comparison test in Graph Pad Prism 9.0 software (San Diego, United States). The value of *p*-value less than 0.05 was considered for significant statistical difference. Compared with the group I–normal ND, the significant difference of the group II–diabetic (STZ) control was symbolized as "#," whereas compared with the diabetic control group II, the significant difference of the groups III, IV, and V (STZ + SME) and group VI–STZ + MET was symbolized as "\*."

#### Results

#### **Bioactive Constituents**

Analysis of SME for bioactive constituents showed the presence of alkaloids, flavonoids, phenols, tannins, and steroids. The percentage yield of freeze-dried SME was found to be 13.50% (w/w).

#### Acute Oral Toxicity Study

No signs of death or toxicity were observed following the ingestion of oral graded doses of SME suspension in 1% (w/v) CMC given for 72 hours. As a result, the OECD recommended a maximum tolerated dose of SME of 2000 mg/kg, and to investigate the potential of SME in STZ-sensitized rats, dosages of 100, 200, and 400 mg/kg were preferred.

Treatment	Body mass (g)	Serum glucose (mg/dL)	Hemoglobin A1c (%)
I—ND	$241.67\pm5.58$	$163.13 \pm 6.75$	$5.04 \pm 0.39$
II—STZ control	$149.17 \pm 3.00^{\#\#}$	$460.10\pm 5.62^{\#\#}$	$12.35 \pm 0.58^{\#\#}$
III—SME (100)	$155.17 \pm 3.00^{ns}$	$446.13 \pm 5.64^{ns}$	$12.21\pm0.50^{ns}$
IV—SME (200)	$170.17 \pm 3.00^{**}$	$424.80 \pm 5.62^{*}$	$10.47 \pm 0.47^{\ast}$
V—SME (400)	$173.17 \pm 3.00^{***}$	$418.8 \pm 5.62^{**}$	$9.61 \pm 0.44^{**}$
VI—MET	$175.67 \pm 5.58^{***}$	$333.13 \pm 6.75^{***}$	$9.41 \pm 0.44^{***}$

Table 1 Effect of SME on body weight, serum glucose, and hemoglobin A1c in diabetic rats

Abbreviations: ANOVA, analysis of variance; ND, nondiabetic; SEM, standard error of mean; SME, Sphaeranthus indicus Linn methanol extract; STZ, streptozotocin.

The data were expressed as mean  $\pm$  SEM (n = 6) and analyzed by one-way ANOVA trailed by *post-hoc* Tukey' multiple comparison test. \*\*\*\*p < 0.001 STZ control versus ND group. <sup>ns</sup> nonsignificant for SME (100), \*p < 0.05 and \*\*\*p < 0.01 for SME (200), \*\*p < 0.01 and \*\*\*\*p < 0.001 for SME (400), and \*\*\*p < 0.001 for SME (200), \*\*p < 0.01 for MET, versus STZ control group.

# Body Weight, Serum Glucose, and Hemoglobin A1c Levels

In the diabetic (STZ) control group II, a substantial fall in body weight and rise in serum glucose and hemoglobin A1c concentration were detected in comparison with the ND group I. A substantial amelioration of reduced body weight and raised glucose and hemoglobin A1c levels was observed in SME-treated groups IV and V and MET-treated group VI, in contrast to the diabetic control group II, at the end of the 56th day (**-Table 1**).

# **Renal Functional Parameters**

Significant inclination in the creatinine, BUN, and uric acid levels was observed in the diabetic (STZ) control group II in contrast to the ND group I. Dose-related declination in the extents of creatinine, BUN, and uric acid was observed in SME-treated groups IV and V and MET-treated group VI, in contrast to the diabetic control group II, at the end of the 56th day (**~Table 2**).

# **Serum Lipid Profile**

In contrast to ND group I, in diabetic (STZ) control group II, there was a modest to significant fall in HDL-C with rises in

TG, TC, LDL-C, and VLDL-C. Moreover, the level of HDL-C was significantly raised, and TG, LDL-C, and VLDL-C levels were significantly lower in SME-treated groups IV and V in contrast to the diabetic (STZ) control group II. There was a significant fall in the level of total TC in SME-treated group V and MET-treated group VI, in contrast to the diabetic control group II, at the end of the 56th day (**~Table 3**).

#### **Oxidative Stress Markers**

After the 56th day of STZ injection, a substantial rise in the extent of MDA production in renal tissue (the index of renal LPO) and a fall in GSH and SOD levels were detected in diabetic (STZ) control group II in contrast to ND group I. In contrast to diabetic (STZ) control group II, SME-treated groups IV and V and MET-treated group VI showed dose-dependent attenuation of raised MDA and reduced GSH and SOD levels in contrast to diabetic (STZ) control groups (**Figs. 1–3**).

# **Renal Histoarchitecture**

ND group I exhibited typical glomeruli with an intact Bowman's capsule, proximal and distal convoluted tubules with normally thickened basement membrane (**Fig. 4A**).

Table 2 Effect of SME on creatinine, blood urea nitrogen (BUN), and uric acid in diabetic rats

Treatment	Creatinine (mg/dL)	BUN (mg/dL)	Uric acid (mg/dL)
I—ND	$0.81\pm0.07$	$16.12 \pm 1.26$	$5.04 \pm 0.60$
II—STZ control	$1.44 \pm 0.06^{\#\#}$	$42.17 \pm 1.03^{\#\#}$	$15.52 \pm 0.53^{\#\#}$
III—SME (100)	$1.31\pm0.06^{ns}$	$39.83 \pm 1.10^{ns}$	$14.82\pm0.53^{ns}$
IV—SME (200)	$1.17 \pm 0.06^{*}$	$36.70 \pm 1.14^{*}$	$13.16\pm0.53^*$
V—SME (400)	$1.12 \pm 0.06^{**}$	$35.53 \pm 1.20^{**}$	$12.74 \pm 0.54^{**}$
VI—MET	$1.06 \pm 0.07^{***}$	$33.33 \pm 1.26^{***}$	$11.56 \pm 0.62^{***}$

Abbreviations: ANOVA, analysis of variance; ND, nondiabetic; SEM, standard error of mean; SME, Sphaeranthus indicus Linn methanol extract; STZ, streptozotocin.

The data were expressed as mean  $\pm$  SEM (n = 6) and analyzed by one-way ANOVA trailed by *post-hoc* Tukey' multiple comparison test. \*\*\*\*p < 0.001 for STZ control versus ND group. <sup>ns</sup> non-significant for SME (100), \*p < 0.05 for SME (200), \*\*p < 0.01 for SME (400), and \*\*\*p < 0.001 for MET, versus STZ control group.

Treatment	Serum lipid profiles (mg/dL)					
	TG	тс	LDL-C	VLDL-C	HDL-C	
I—ND	$147.00\pm11.22$	$149.57\pm5.73$	$57.67 \pm 4.34$	$29.40 \pm 2.24$	$62.50\pm2.83$	
II—STZ control	$316.83 \pm 7.91^{\#\#}$	$282.20 \pm 7.43^{\#\#}$	$187.83 \pm 5.88^{\#\#}$	$63.37 \pm 1.34^{\#\#}$	$31.00 \pm 2.38^{\#\#}$	
III—SME (100)	$297.83 \pm 7.96$ <sup>ns</sup>	$283.90 \pm 6.38 \ ^{ns}$	$186.17 \pm 4.96^{ns}$	$59.57 \pm 1.59 \ ^{\text{ns}}$	$38.17\pm2.50^{ns}$	
IV—SME (200)	$276.83 \pm 9.16^{*}$	$265.53 \pm 5.34 \ ^{ns}$	$167.67 \pm 4.33^{*}$	$55.37 \pm 1.83^{\ast}$	$42.50\pm2.68^*$	
V—SME (400)	$268.83 \pm 8.79^{**}$	$262.27 \pm 5.11^{\ast}$	$163.00 \pm 4.03^{**}$	$53.77 \pm 1.76^{**}$	$45.50 \pm 2.68^{**}$	
VI-MET	$178.17 \pm 11.39^{***}$	$235.30 \pm 6.06^{***}$	$151.00 \pm 4.12^{***}$	$35.63 \pm 2.28^{***}$	$48.67 \pm 2.54^{***}$	

Table 3 Effect of SME on serum lipid profiles (TG, TC, LDL-C, VLDL-C, and HDL-C) in STZ sensitized diabetic rats

Abbreviations: ANOVA, analysis of variance; HDL-C, VLDL-C, high-density lipoprotein-cholesterol; ND, nondiabetic; SEM, standard error of mean; SME, *Sphaeranthus indicus* Linn methanol extract; STZ, streptozotocin; TC, total cholesterol; TG, triglycerides; VLDL-C, very low-density lipoprotein-cholesterol. The data were expressed as mean  $\pm$  SEM (n = 6) and analyzed by one-way ANOVA trailed by *post-hoc* Tukey' multiple comparison test. \*\*\*p < 0.001 for the STZ control versus ND group. <sup>ns</sup> nonsignificant for SME (100), \*p < 0.05 for SME (200) except for TC, \*p < 0.05 and \*\*p < 0.01 for SME (400), and \*\*\*p < 0.001 for MET, versus STZ control group.



**Fig. 1** Effect of SME on diabetes-induced alterations in the extent of MDA production in renal tissue. The data were expressed as mean  $\pm$  SEM (n = 6) and analyzed by one-way analysis of variance trailed by post-hoc Tukey' multiple comparison test. <sup>###</sup>p < 0.001 for STZ control versus ND group. <sup>ns</sup> nonsignificant for SME (100), \*p < 0.05 for SME (200), \*\*p < 0.01 for SME (400), and \*\*\*p < 0.001 for MET, versus STZ control group. MDA, malondialdehyde; ND, nondiabetic; SEM, standard error of mean; SME, *Sphaeranthus indicus* Linn methanol extract; STZ, streptozotocin.



**Fig. 2** Effect of SME on diabetes-induced alterations in GSH level in renal tissue. The data were expressed as mean  $\pm$  SEM (n = 6) and analyzed by one-way analysis of variance trailed by post-hoc Tukey' multiple comparison test. ###p < 0.001 for STZ control versus ND group. <sup>ns</sup> non-significant for SME (100), \*p < 0.05 for SME (200), \*\*p < 0.01 for SME (400) and MET, versus STZ control group. GSH, reduced glutathione; ND, nondiabetic; SEM, standard error of mean; SME, *Sphaeranthus indicus* Linn methanol extract; STZ, streptozotocin.



**Fig. 3** Effect of SME on diabetes-induced alterations in SOD level in renal tissue. The data were expressed as mean  $\pm$  SEM (n = 6) and analyzed by one-way analysis of variance trailed by post-hoc Tukey' multiple comparison test. ###p < 0.001 for STZ control versus ND group. <sup>ns</sup> nonsignificant for SME (100), \*p < 0.05 for SME (200), \*p < 0.01 for SME (400), and \*\*\*p < 0.001 for MET, versus STZ control group. SOD, superoxide dismutase; ND, nondiabetic; SEM, standard error of mean; SME, *Sphaeranthus indicus* Linn methanol extract; STZ, streptozotocin.

Diabetic (STZ) control group II showed dilated Bowman's capsule, congested and atrophied glomeruli with enlarged degenerated tubules, and inflamed interstitial tissue (**>Fig. 4B**). Significant attenuation of renal lesions in SME (200), SME (400), and MET (500) treated rats was characterized by a reduction in vacuolar degeneration of tubules, reduced Bowman's space, and glomerular congestion (**>Fig. 4D-F**). Restoration of renal architecture was insignificant in SME (100) treated rats.

# Discussion

Diabetes is a complicated endocrine disorder that involves an insulin deficiency and chronic hyperglycemia with abnormal protein, lipid, and carbohydrate metabolism. By 2025, it is predicted that more than 300 million individuals will be affected by diabetes worldwide.<sup>32</sup> A major late-onset systemic consequence of diabetes is DNP. End-stage renal failure is most frequently caused by DM. DNP affects approximately 15 to 25% of people with type 1 and 30 to 40% of people with



**Fig. 4** (A–F) Effect of chronic treatment of SME on renal histoarchitecture in STZ-induced diabetic nephropathy. Photomicrographs of sections of kidneys from rats stained with hematoxylin and eosin. (A) ND group I, (B) STZ control group II, (C) SME (100) group III, (D) SME (200) group IV, (E) SME (400) group V, and (F) MET group VI. Microscopic examination under  $400 \times$  light microscopy. G, glomeruli; GS, glomerular space; ND, nondiabetic; SME, *Sphaeranthus indicus* Linn methanol extract; STZ, streptozotocin; T, tubules; VD, vacuolar degeneration.

type 2 diabetes.<sup>33</sup> Although the existing drugs reduce the development of DNP, there is increased interest in the use of natural treatments to delay the onset of this problem. The use of natural products and functional meals as worthwhile alternatives for the creation of innovative antidiabetics and reno-protectives has recently become the focus of various research studies.<sup>34–36</sup>

The current work might be interesting to evaluate the renoprotective ability of *Sphaeranthus indicus* Linn to ameliorate DNP in STZ-sensitized Wistar albino rats because of the plant's well-established antioxidant, antihyperglycemic, antihyperlipidemic, and renoprotective profiles and the presence of bioactive phytoconstituents such as eudesmanolides, eudesmanolides, sesquiterpene lactone, sterol glycoside, flavanoids, and essential oil.<sup>17</sup>

STZ is an antibiotic with a wide spectrum of action that is derived from Streptomyces achromogenes. Since it was established that STZ possesses diabetogenic effects that are mediated by pancreatic cell death, STZ-induced type 1 diabetes in experimental animals has become a well-liked experimental paradigm. A growing body of research shows that STZ produces an excess of oxidants, including hydrogen peroxide and oxygen-containing free radicals like the superoxide radical anion and the hydroxyl radical. DNA damage was brought on in pancreatic  $\beta$ -cells by the alkylation with carbonium ions generated by methyldiazohydroxide.<sup>37,38</sup>

There is a significant reduction in body weight due to hyperglycemia, hypoinsulinemia, increased muscle wasting, and loss of tissue proteins in STZ-induced diabetes. Dehydration and the breakdown of lipids and proteins are two possible causes of the diabetic rat's body weight loss. Increased catabolic processes that result in muscle loss may potentially be a factor in diabetic rats' impaired ability to acquire weight. Additionally, because of its anabolic action, increased insulin secretion may also lead to increased protein synthesis.<sup>39-41</sup>

Hemoglobin A1c is gradually raised in diabetes as a result of chronic hyperglycemia resulting from the interaction between blood glucose and hemoglobin. According to the available data, glycation may lead diabetics to produce free radicals that are obtained from oxygen, which may be the main factor causing DNP.<sup>42,43</sup> Following the 4-week treatment of SME and metformin in diabetic rats, significant and dose-dependent mitigation of lowered body weight as well as incremental blood glucose and hemoglobin A1c levels was seen in this study.

The main contributing factors to DNP include albuminuria, mesangial matrix enlargement, and moderate tubulointerstitial disease, as well as increased plasma creatinine, BUN, and uric acid levels.<sup>44,45</sup> The treatment of SME in STZ-sensitized diabetic rats in this study resulted in dosedependent improvement in the elevated levels of blood creatinine, BUN, and uric acid. Contrary to diabetic (STZ) control rats, metformin dramatically reduced the levels of blood creatinine, BUN, and uric acid.

Hyperlipidemia is another documented consequence of non-insulin dependent diabetes mellitus (NIDDM) that coexists with hyperglycemia and is characterized by higher levels of cholesterol, TGs, and phospholipids, as well as alterations in lipoproteins.<sup>46,47</sup> The increased prevalence of atherosclerotic disease, a significant cause of early mortality in diabetic patients, has sparked interest in the study of plasma lipids in diabetes.<sup>48</sup> Several biochemical pathways have been postulated to explain diabetic hyperlipidemia. One of these is the increased functioning of hormone-sensitive lipase, which catalyzes the migration of fatty acids from triacylglycerols deposited in adipose tissues. As a result, the larger the number of fatty acids returning to the liver, the more triacylglycerols are formed and produced in VLDL. Hyperlipidemia in diabetes has also been linked to a decrease in the activity of lipoprotein lipase, an enzyme attached to endothelial cells that catalyzes the breakdown of triacylglycerols in VLDL and chylomicrons.<sup>49-51</sup> In this study, an altered serum lipid profile in STZ-sensitized diabetic rats was restored significantly following SME treatment.

Numerous diabetes problems, including DNP, are thought to have their origins in the creation of ROS that is triggered by hyperglycemia. ROS weaken the cell's antioxidant defenses, making it more vulnerable to oxidative damage. After that, it oxidizes proteins, lipids, and DNA, changing the way cells behave and function. Both for maintaining the plasma's antioxidant state and as a free radical scavenger, GSH is crucial. Using SOD, hydrogen peroxide, a less reactive ROS, is converted from superoxide to hydrogen peroxide, which catalase subsequently reduces to water. Consequently, catalase helps SOD completely neutralize ROS. MDA, a by-product of advanced lipid oxidation, serves as an excellent biomarker for lipid peroxidation brought on by free radicals. Plasma, proximal tubule cells, renal cortex, and mesangial cells all had increased MDA concentrations.<sup>52,53</sup> In diabetic rats exposed to STZ, SME prevented elevated MDA levels. The fact that SME treatment increased GSH and SOD concentrations in STZ-diabetic rats further supports its antioxidant strength.

Furthermore, evidence of SME-mediated nephroprotection can be found in the restoration of renal histoarchitecture, which is characterized by a significant attenuation of lesions like vacuolar degeneration of tubules, reduced Bowman's space, and glomerular congestion.

# Conclusion

In this study, administration of SME in diabetic rats markedly reduced STZ-induced increases in the levels of blood glucose, hemoglobin A1c levels, oxidative stress, renal functional and serum lipid parameters, and repaired the histopathological damage to the kidney. The administration of the SME enhanced the antioxidant capability by increasing GSH and SOD levels, and reducing MDA levels in the STZ-treated diabetic group. In summary, antihyperglycemic, antioxidant, and antihyperlipidemic potentials of SME afford nephroprotection against STZ-induced DNP. Future studies are essential to isolate, characterize, and elaborate the mechanism of action of the bioactive constituents of the SME in providing nephroprotection.

# **Ethics Approval**

The protocol No. 24/BNCP/IAEC/2021 of the experiment was approved by the Institutional Animal Ethics Committee (IAEC) of B. N. College of Pharmacy, Bhupal Nobles' University, Udaipur-313001, Rajasthan (India) (870/PO/Re/S/05/CPCSEA).

## Availability of Data and Materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

#### Authors' Contributions

All authors contributed to the study conception and design. Experimental studies were conducted by VBJ. Data collection and analysis were performed by VBJ and JSV. The first draft of the manuscript was written by VBJ under the supervision of JSV. Both the authors commented on previous versions of the manuscript. Both the authors read and approved the final manuscript.

#### Funding

None.

Conflict of Interests None declared.

#### Acknowledgments

The authors are thankful to the Department of Pharmacology, B. N. College of Pharmacy, B. N. University, Udaipur-313001, Rajasthan (India), for the infrastructure facilities needed to carry out this work. The authors further expressed sincere gratitude to Dr. Hemantkumar A. Thakur, Head of Postgraduate Department of Botany, GES's HPT Arts and RYK Science Institution, Nashik, Maharashtra, India, for plant authentication.

#### References

- Berezin A. Metabolic memory phenomenon in diabetes mellitus: achieving and perspectives. Diabetes Metab Syndr 2016;10(2, Suppl 1):S176–S183
- 2 Kaur N, Kishore L, Singh R. Dillenia indica L. attenuates diabetic nephropathy via inhibition of advanced glycation end products accumulation in STZ-nicotinamide induced diabetic rats. J Tradit Complement Med 2017;8(01):226–238
- 3 Nathan DM, Davidson MB, DeFronzo RA, et al; American Diabetes Association. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes Care 2007;30(03):753–759
- 4 Wendt T, Tanji N, Guo J, et al. Glucose, glycation, and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. J Am Soc Nephrol 2003;14(05):1383–1395
- 5 Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev 2013;93(01):137–188
- 6 Gonzalez Suarez ML, Thomas DB, Barisoni L, Fornoni A. Diabetic nephropathy: is it time yet for routine kidney biopsy? World J Diabetes 2013;4(06):245–255
- 7 Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 2008;57(06): 1446–1454
- 8 Nath KA, Norby SM. Reactive oxygen species and acute renal failure. Am J Med 2000;109(08):665–678

- 9 Vallon V, Thomson SC. Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. Annu Rev Physiol 2012;74:351–375
- 10 Singh R, Devi S, Gollen R. Role of free radical in atherosclerosis, diabetes and dyslipidaemia: larger-than-life. Diabetes Metab Res Rev 2015;31(02):113–126
- 11 Jha JC, Banal C, Chow BS, Cooper ME, Jandeleit-Dahm K. Diabetes and kidney disease: role of oxidative stress. Antioxid Redox Signal 2016;25(12):657–684
- 12 Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology 2005;15(07):16R–28R
- 13 Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin Exp Med 2007;7(01):24–29
- 14 Han H, Cao A, Wang L, et al. Huangqi decoction ameliorates streptozotocin-induced rat diabetic nephropathy through antioxidant and regulation of the TGF-β/MAPK/PPAR-γ signaling. Cell Physiol Biochem 2017;42(05):1934–1944
- Venkatesh S, Reddy GD, Reddy BM, Ramesh M, Rao AV. Antihyperglycemic activity of Caralluma attenuata. Fitoterapia 2003;74(03): 274–279
- 16 Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, Andrade-Sierra J. Oxidative stress in diabetic nephropathy with early chronic kidney disease. J Diabetes Res 2016; 2016:7047238
- 17 Galani VJ, Patel BG, Rana DG. Sphaeranthus indicus Linn.: a phytopharmacological review. Int J Ayurveda Res 2010;1(04): 247–253
- 18 Ramachandran S. Review on Sphaeranthus indicus Linn. (Koțt aikkarantai). Pharmacogn Rev 2013;7(14):157–169
- 19 Srinivasan VM, Jessy KK, Anand Alex EM. Effect of Sphaeranthus indicus Linn. On Gentamicin induced acute renal failure in rats. Indian J Pharmacol 2008;40:71
- 20 Mathew JE, Mantri A, Vachala SD, Srinivasan KK, Movaliya V. Effect of Sphaeranthus indicus ethanol extract on tissue antioxidant activity in gentamicin induced nephrotoxic rats. Herba Pol 2009;55(04):86–95
- 21 Mathew JE, Joseph A, Srinivasan K, Dinakaran SV, Mantri A, Movaliya V. Effect of ethanol extract of Sphaeranthus indicus on cisplatin-induced nephrotoxicity in rats. Nat Prod Res 2012;26 (10):933–938
- 22 Harborne JB. Phytochemical Methods. London: Chapman and Hall; 1973:49–188
- 23 Compendium of CPCSEA Committee for the purpose of control and supervision of experiments on animals. Ministry of Environment, Forest and Climate Change. Government of India; 2008:1–213
- 24 Organisation for Economic Co-operation and Development Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. OECD Publishing; 2008
- 25 Choudhary GP, Jain AP. Evaluation of acute, subacute and LD50 values of methanolic extract of Sphaeranthus indicus leaves in Albino mice. Res J Pharm Technol 2021;14(05):2487–2492
- 26 Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia 2012;83(04):650–659
- 27 Navale AM, Paranjape A. Antidiabetic and renoprotective effect of Anogeissus acuminata leaf extract on experimentally induced diabetic nephropathy. J Basic Clin Physiol Pharmacol 2018;29 (04):359–364
- 28 Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharm Biol 2014;52(07): 814–828

- 29 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95 (02):351–358
- 30 Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82(01):70–77
- 31 Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247(10):3170–3175
- 32 Standl E, Khunti K, Hansen TB, Schnell O. The global epidemics of diabetes in the 21st century: current situation and perspectives. Eur J Prev Cardiol 2019;26(2\_suppl, suppl):7–14
- 33 Koye DN, Magliano DJ, Nelson RG, Pavkov ME. The global epidemiology of diabetes and kidney disease. Adv Chronic Kidney Dis 2018;25(02):121–132
- 34 Governa P, Baini G, Borgonetti V, et al. Phytotherapy in the management of diabetes: a review. Molecules 2018;23(01):105
- 35 Shukla R, Banerjee S, Tripathi YB. Antioxidant and antiapoptotic effect of aqueous extract of Pueraria tuberosa (Roxb. Ex Willd.) DC. On streptozotocin-induced diabetic nephropathy in rats. BMC Complement Altern Med 2018;18(01):156
- 36 Borgohain MP, Chowdhury L, Ahmed S, et al. Renoprotective and antioxidative effects of methanolic Paederia foetida leaf extract on experimental diabetic nephropathy in rats. J Ethnopharmacol 2017;198:451–459
- 37 Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protocols Pharmacol 2015;70:47.1, 20
- 38 Wilson GL, Leiter EH. Streptozotocin interactions with pancreatic beta cells and the induction of insulin-dependent diabetes. Curr Top Microbiol Immunol 1990;156:27–54
- 39 Zari TA, Al-Attar AM. Effects of ginger and clove oils on some physiological parameters in streptozotocin-diabetic and nondiabetic rats. J Med Sci 2007;7:267–275
- 40 Jayaprasad B, Sharavanan PS, Sivaraj R. Effect of Chloroxylon swietenia Dc bark extracts on STZ induced diabetic rats with special attention to its glycoprotein levels. Pharm Lett 2015; 7:414–418
- 41 Zhang Y, Feng F, Chen T, Li Z, Shen QW. Antidiabetic and antihyperlipidemic activities of Forsythia suspensa (Thunb.) Vahl (fruit) in streptozotocin-induced diabetes mice. J Ethnopharmacol 2016;192:256–263
- 42 Lin CC, Chen CC, Chen FN, et al. Risks of diabetic nephropathy with variation in hemoglobin A1c and fasting plasma glucose. Am J Med 2013;126(11):1017.e1–1017.e10
- 43 Niveditha H, Yogitha C, Liji P, et al. Clinical correlation of HbA1c and diabetic nephropathy with diabetic retinopathy. J Evol Med Dent Sci 2013;2(49):9430
- 44 Ju Y, Su Y, Chen Q, et al. Protective effects of Astragaloside IV on endoplasmic reticulum stress-induced renal tubular epithelial cells apoptosis in type 2 diabetic nephropathy rats. Biomed Pharmacother 2019;109:84–92
- 45 Abdel-Wahab AF, Bamagous GA, Al-Harizy RM, et al. Renal protective effect of SGLT2 inhibitor dapagliflozin alone and in combination with irbesartan in a rat model of diabetic nephropathy. Biomed Pharmacother 2018;103:59–66
- 46 Sheu WH, Jeng CY, Lee WJ, Lin SY, Pei D, Chen YT. Simvastatin treatment on postprandial hypertriglyceridemia in type 2 diabetes mellitus patients with combined hyperlipidemia. Metabolism 2001;50(03):355–359
- 47 Bagdade JD, Helve E, Taskinen MR. Effects of continuous insulin infusion therapy on lipoprotein surface and core lipid composition in insulin-dependent diabetes mellitus. Metabolism 1991;40 (05):445–449
- 48 Betteridge DJ. Diabetes, lipoprotein metabolism and atherosclerosis. Br Med Bull 1989;45(01):285-311
- 49 Zeid IEMEA, Jaghthmi OHAA. Hypoglycemic and hypolipidemic effects of two mangrove plants in a streptozotocin-induced animal model of diabetes. J Adv Vet Anim Res 2020;7(03):421–428

- 50 Reynisdottir S, Angelin B, Langin D, et al. Adipose tissue lipoprotein lipase and hormone-sensitive lipase. Contrasting findings in familial combined hyperlipidemia and insulin resistance syndrome. Arterioscler Thromb Vasc Biol 1997;17(10):2287–2292
- 51 Shawky S, Abdel-Aziz SA, Amer HA, Hegazy II, Hussein MA. Biochemical effects of some trace elements in experimental diabetic rats. *Oct. 6 Univ.* J Med Sci 2018;4:14–22
- 52 Nandini HS, Naik PR. Antidiabetic, antihyperlipidemic and antioxidant effect of Vincamine, in streptozotocin-induced diabetic rats. Eur J Pharmacol 2019;843:233–239
- 53 Papachristoforou E, Lambadiari V, Maratou E, Makrilakis K. Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. J Diabetes Res 2020;2020:7489795