Article history
Received: August 9, 2026
Revised: August 27, 2026
Accepted: September 3, 2026
Published: September 15, 2026

Unicross journal of Science, Engineering & Technology (UJOSET), Journal of the Faculty of Physical Sciences, University of Cross River State
VOL 1 ISSUE 2, 2026

Article Type: Research

Antihyperglycaemic, Antioxidant, and Nephroprotective Effects of Ethanol Seed Extract of *Silybum marianum* (L.) Gaertn. in Streptozotocin-Induced Diabetic Wistar Rats

Bayim, P-R. Bayim[1], David Effiong Bassey*[1], & Michael, Mayehm Mbom[1]

Department of Biochemistry University of Cross River State, Calabar, Nigeria
*Corresponding to davidbassey27253@gmail.com

Abstract

Diabetic nephropathy (DN) is a leading cause of chronic kidney disease worldwide, with oxidative stress playing a central role in its pathogenesis. *Silybum marianum* (milk thistle) possesses established antioxidant and hepatoprotective properties, but its concurrent antihyperglycaemic, antioxidant, and nephroprotective effects in experimental diabetes remain incompletely characterised. This study evaluated the antihyperglycaemic, antioxidant, and nephroprotective effects of ethanol seed extract of *Silybum marianum* (SMESE) in streptozotocin (STZ)-induced diabetic Wistar rats. Thirty male Wistar rats (150–200 g) were randomly assigned to five groups (n=6): normal control, diabetic control, SMESE 200 mg/kg, SMESE 400 mg/kg, and metformin 100 mg/kg. Diabetes was induced by a single intraperitoneal STZ injection (60 mg/kg). Treatments were administered orally once daily for 28 days. Fasting blood glucose, serum insulin, renal function markers (creatinine, urea, total protein), antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], reduced glutathione [GSH]), and lipid peroxidation (malondialdehyde [MDA]) were assessed. Histopathological examination of kidney tissues was performed. SMESE demonstrated an LD50 >2900 mg/kg, indicating practical non-toxicity. Phytochemical screening revealed abundant flavonoids (+++) and phenolics (+++), with moderate tannins (++) and terpenoids (++). STZ-induced diabetic rats exhibited significant hyperglycaemia (17.6 ± 1.4 mmol/L), reduced serum insulin (5.8 ± 0.9 µIU/mL), elevated creatinine (1.45 ± 0.12 mg/dL) and urea (48.6 ± 3.2 mg/dL), depressed antioxidant enzymes (SOD: 11.3 ± 1.4 U/mg; CAT: 7.2 ± 0.9 µmol H_2O_2/min/mg; GSH: 18.6 ± 2.1 µmol/mg), and elevated MDA (9.6 ± 0.8 nmol/mg). SMESE treatment produced dose-dependent improvements: the high-dose group (400 mg/kg) reduced fasting blood glucose to 8.1 ± 0.9 mmol/L, restored serum insulin to 13.1 ± 1.4 µIU/mL, reduced creatinine to 0.85 ± 0.09 mg/dL and urea to 22.8 ± 2.1 mg/dL, enhanced antioxidant enzyme activities (SOD: 23.4 ± 1.8 U/mg; CAT: 14.8 ± 1.3 µmol H_2O_2/min/mg; GSH: 35.7 ± 2.6 µmol/mg), and reduced MDA to 4.2 ± 0.5 nmol/mg. Histopathological findings confirmed preservation of renal architectural integrity, with outcomes approaching those of metformin. SMESE exhibits significant antihyperglycaemic, antioxidant, and nephroprotective effects in STZ-induced diabetic rats. The extract demonstrates safety and efficacy approaching metformin, suggesting its potential as a plant-derived adjunctive therapy for diabetic nephropathy. Further mechanistic and chronic toxicity studies are warranted before translational application.

Keywords: *Silybum marianum*, silymarin, streptozotocin, antioxidant, nephroprotective, antihyperglycaemic

Cite this article as: Bayim, P-R. Bayim, David Effiong Bassey, & Michael, Mayehm Mbom (2026). Antihyperglycaemic, Antioxidant, and Nephroprotective Effects of Ethanol Seed Extract of *Silybum marianum* (L.) Gaertn. in Streptozotocin-Induced Diabetic Wistar Rats. *Unicross journal of Science, Engineering & Technology* ***1****(2) pp: 58-76.*

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association (ADA, 2023). The disease has emerged as one of the most significant public health challenges of the twenty-first century, with its prevalence increasing at an alarming rate globally. The International Diabetes Federation (IDF, 2021) estimated that 537 million adults aged 20 to 79 years were living with diabetes in 2021, representing 10.5% of the global adult population. This figure is projected to rise to 643 million by 2030 and 783 million by 2045, with the most dramatic increases anticipated in low- and middle-income countries, particularly in sub-Saharan Africa (IDF, 2021; Ogurtsova *et al*., 2017). In Africa, approximately 24 million adults were affected by diabetes in 2021, and Nigeria ranks among the countries with the highest prevalence on the continent (IDF, 2021). The escalating burden of diabetes in Nigeria is attributable to rapid urbanisation, nutritional transitions towards Westernised dietary patterns, increasing sedentary lifestyles, and the growing prevalence of obesity (Akinluge, 2003). Diabetes-related mortality is substantial, with the IDF estimating 6.7 million deaths attributable to diabetes globally in 2021, while global diabetes-related health expenditure reached USD 966 billion in the same year.

Persistent hyperglycaemia exerts lethal effects on multiple organ systems through several interconnected pathophysiological mechanisms, including the polyol pathway, protein kinase C activation, advanced glycation end-product (AGE) formation, and, most critically, the induction of oxidative stress (Brownlee, 2001; Giacco & Brownlee, 2010). Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and the capacity of endogenous antioxidant defence systems to neutralise them, culminating in lipid peroxidation, protein oxidation, DNA damage, and consequent cellular dysfunction and tissue injury (Okpashi *et al*., 2014; Obi *et al.,* 2016). The kidneys are particularly vulnerable to diabetic injury owing to their rich blood supply, high metabolic activity, and the unique haemodynamic environment of the glomerular microcirculation. Diabetic nephropathy (DN) is the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) globally, affecting approximately 20–40% of all individuals with diabetes (Umanath & Lewis, 2018). Clinically, DN is characterised by progressive proteinuria, declining glomerular filtration rate (GFR), hypertension, and ultimately renal failure, all underpinned by oxidative stress-driven pathological processes at the cellular level (Forbes *et al*., 2008; Yaribeygi *et al*., 2020).

Current pharmacological management of diabetes relies primarily on insulin therapy and synthetic oral hypoglycaemic agents such as metformin, sulfonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors, and sodium-glucose cotransporter-2 (SGLT-2) inhibitors. Metformin, a biguanide derivative, remains the first-line pharmacological agent for the management of type 2 diabetes mellitus (T2DM) due to its well-established efficacy, safety profile, and cardiovascular protective effects (ADA, 2023). However, while these agents provide meaningful glycaemic control, they are associated with significant adverse effects, limited accessibility in resource-constrained settings, and do not adequately address the underlying oxidative mechanisms of diabetic complications (Okpashi *et al*., 2014). Consequently, there is a compelling scientific rationale for investigating plant-derived compounds with concurrent hypoglycaemic, antioxidant, and nephroprotective properties. *Silybum marianum* (L.) Gaertn., commonly known as milk thistle, is among the most extensively studied medicinal plants worldwide (Abenavoli *et al*., 2018). The plant is native to the Mediterranean region but is now widely distributed across Europe, Asia, and Africa. Its seeds contain a complex of flavonoids collectively designated as silymarin, of which silybin (also known as silibinin) is the most abundant constituent, accounting for approximately 50–70% of total silymarin content (Gazak *et al*., 2007; Abenavoli *et al*., 2018). Silymarin has been rigorously investigated for its hepatoprotective properties, but accumulating evidence also supports its antioxidant, anti-inflammatory, antifibrotic, and insulin-sensitising effects that are relevant to the prevention and management of diabetic

complications (Huseini *et al*., 2006; Navarro-González *et al*., 2011; MacDonald-Ramos *et al*., 2021). The antioxidant activity of silymarin is mediated through multiple mechanisms: direct free-radical scavenging, metal chelation, and upregulation of endogenous antioxidant enzymes (SOD, CAT, GPx) through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway (Gazak *et al*., 2007; Bai *et al*., 2016; Awla *et al*., 2023). Furthermore, silymarin has been shown to enhance insulin sensitivity through activation of the IRS-1/PI3K/Akt signalling axis and upregulation of GLUT-4 expression (Soto *et al*., 2010).

Streptozotocin (STZ) is the most widely employed pharmacological agent for the induction of experimental diabetes in rodents (Eleazu *et al*., 2013). Despite the extensive pharmacological literature on *Silybum marianum*, few studies have simultaneously and systematically evaluated its antioxidant capacity, nephroprotective effects, and antihyperglycaemic properties in the STZ-induced rat model. This study was therefore designed to address this gap by comprehensively examining these outcomes in experimentally diabetic albino rats, utilising metformin as the standard reference drug for comparative purposes given its established antioxidant and nephroprotective properties in addition to its antihyperglycaemic effects (Obi *et al*., 2016).

Diabetic nephropathy remains a major cause of morbidity and mortality in diabetic patients, with current therapeutic options limited by incomplete efficacy, adverse effects, and limited accessibility in resource-constrained settings. There is a need for safe, effective, and affordable alternative or adjunctive therapies. *Silybum marianum* has demonstrated promising antioxidant and organ-protective properties, but comprehensive evaluation of its concurrent antihyperglycaemic, antioxidant, and nephroprotective effects in experimental diabetes, particularly with comparison to standard therapy, is lacking. This research aimed to evaluate the antihyperglycaemic, antioxidant, and nephroprotective effects of ethanol seed extract of *Silybum marianum* (L.) Gaertn. in streptozotocin-induced diabetic albino rats.

Materials and Methods

Study Design

This study was an experimental laboratory investigation employing a completely randomised design (CRD). Thirty male Wistar rats were randomly assigned to five experimental groups (n=6 per group) using a computer-generated random number sequence. The study was conducted over 28 days of treatment following confirmation of diabetes induction. All biochemical analyses and histopathological assessments were performed by investigators blinded to group assignments.

Collection of Seeds and Authentication

Fresh, ripe seeds of *Silybum marianum* were harvested from a cultivated botanical garden in Calabar South Local Government Area, Cross River State, Nigeria, during the dry season (January–February 2025). The plant was authenticated by a plant taxonomist at the Forest Herbarium, UNICROSS Botanical Garden, Calabar, where a voucher specimen was deposited and assigned Voucher Number FHI 112361. The seeds were collected from healthy, mature plants showing no evidence of disease or pest infestation. The harvested seeds were thoroughly washed under running tap water to remove surface debris, followed by a final rinse with distilled water. They were then shade-dried at ambient temperature (25 ± 2°C) for three weeks in a well-ventilated laboratory space to prevent photodegradation of phytochemicals. The completely dried seeds were pulverised into a coarse powder using an electric mechanical grinder (Thomas-Wiley® Mill, Model 4), and the resulting powder was stored in airtight amber glass containers at room temperature until extraction.

Preparation of Ethanol Seed Extract of *Silybum marianum*

Ethanol extraction was employed as it is the most effective solvent for solubilising the flavonolignan constituents of *Silybum marianum* seeds, including silybin and related compounds. Exactly 500 g of the powdered seed material was macerated in 2.5 litres of

absolute ethanol (99.9%) at a ratio of 1:5 (w/v) in an amber glass container. The mixture was covered and allowed to macerate at room temperature (25 ± 2°C) for 72 hours with intermittent shaking every 12 hours to enhance extraction efficiency. The macerate was first filtered through a double-layered muslin cloth, and the marc (spent plant material) was pressed to recover residual extract. The filtrate was subsequently passed through Whatman No. 1 filter paper to obtain a clear filtrate.

The clear filtrate was concentrated under reduced pressure using a rotary evaporator (Büchi R-300) at 40°C to remove approximately 70% of the ethanol. The resultant semi-solid concentrate was transferred into pre-weighed glass Petri dishes and lyophilised using a freeze dryer (Labconco FreeZone 2.5) to yield a dry, hygroscopic extract powder. The percentage yield of the extract was calculated using Equation 1:

Equation 1: Percentage Yield

$$\text{Percentage Yield (\%)} = \frac{\text{Mass of dry extract (g)}}{\text{Mass of powdered seed material (g)}} \times 100$$

The extract (henceforth referred to as SMESE, *Silybum marianum* Ethanol Seed Extract) was stored in airtight, light-proof containers at -20°C until required for administration. All extractions were performed in triplicate to ensure consistency, and the average yield was reported.

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of the SMESE was conducted using standard procedures to identify the classes of phytochemicals present (Harborne, 1998; Trease & Evans, 2002). The following constituents were screened:

Alkaloids

Mayer's and Dragendorff's tests were performed. For Mayer's test, the extract was treated with Mayer's reagent (potassium mercuric iodide); the formation of a creamy white precipitate indicated the presence of alkaloids. For Dragendorff's test, the extract was treated with Dragendorff's reagent (potassium bismuth iodide); the formation of an orange-red precipitate indicated the presence of alkaloids.

Flavonoids

The alkaline reagent test was performed by treating the extract with a few drops of 10% sodium hydroxide solution; the formation of an intense yellow colour that disappeared upon addition of dilute hydrochloric acid indicated the presence of flavonoids. Additionally, the Shinoda test was performed by adding magnesium ribbon and concentrated hydrochloric acid to the extract; the development of a pink, red, or orange colour indicated the presence of flavonoids.

Tannins

The lead acetate precipitation test was performed by adding 2 mL of 10% lead acetate solution to the extract; the formation of a white precipitate indicated the presence of tannins. Additionally, the ferric chloride test was performed by adding a few drops of 5% ferric chloride solution to the extract; the development of a blue-black or green colour indicated the presence of tannins.

Saponins

The foam test was performed by shaking 2 mL of the extract with 5 mL of distilled water in a test tube; the formation of persistent foam (for at least 15 minutes) indicated the presence of saponins.

Steroids and Sterols

The Liebermann-Burchard test was performed by dissolving the extract in chloroform, followed by the addition of acetic anhydride and concentrated sulphuric acid; the development of a blue-green colour indicated the presence of steroids.

Terpenoids

The Salkowski test was performed by adding 2 mL of chloroform to the extract, followed by the careful addition of 3 mL of concentrated sulphuric acid; the formation of a reddish-brown colour at the interface indicated the presence of terpenoids.

Cardiac Glycosides

The Keller-Killiani test was performed by dissolving the extract in 2 mL of glacial acetic acid containing one drop of ferric chloride solution, followed by the addition of 1 mL of

concentrated sulphuric acid; the formation of a brown ring at the interface indicated the presence of cardiac glycosides.

Phenolic Compounds

The ferric chloride test was performed by adding a few drops of 5% ferric chloride solution to the extract; the development of a blue-black or green colour indicated the presence of phenolic compounds. All tests were performed in triplicate, and results were recorded as positive (+) or negative (-) based on characteristic colour changes or precipitate formation. The relative abundance of phytochemicals was scored semi-quantitatively as follows: +++ (highly abundant), ++ (moderately abundant), + (present in low concentration), and - (absent).

Acute Oral Toxicity Study (LD50 Determination)

The acute oral toxicity of SMESE was evaluated using the Lorke (1983) method, which is a widely accepted and ethically sound approach for the determination of median lethal dose (LD50) in rodent models. The LD50 is defined as the dose of a substance that is lethal to 50% of a test population under specified conditions of administration. The Lorke (1983) method was employed in two sequential phases, each using healthy adult male albino rats.

Phase I (Range-Finding Study)

Nine rats were divided into three groups of three animals each. They were administered single oral doses of SMESE at 10, 100, and 1000 mg/kg body weight, respectively, by oral gavage. All animals were observed continuously for the first four hours and thereafter at 24-hour intervals for 14 days. Behavioural changes, signs of toxicity, and mortality were recorded.

Phase II (Definitive Study)

Based on the Phase I results, where no mortality was recorded at any dose level, three additional groups of three animals each were administered higher doses: 1600, 2900, and 5000 mg/kg body weight. Mortality and behaviour were monitored over a further 14 days.

The LD50 was calculated using Equation 2:

Equation 2: LD50 Calculation

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

where D_0 represents the highest dose at which no mortality occurred, and D_{100} represents the lowest dose at which all animals died. If 100% mortality was not achieved, the LD50 was reported as greater than the highest dose tested with no mortality.

Experimental Animals and Ethical Considerations

Thirty (30) healthy, young adult male albino rats (*Rattus norvegicus*, Wistar strain), weighing between 150 and 200 g, were procured from the Animal House Facility of the Department of Biochemistry, University of Cross River State. Male rats were used exclusively to eliminate hormonal variability associated with the oestrous cycle in females, which could confound biochemical and physiological outcome measures. The rats were housed in well-ventilated, sanitised polypropylene cages with sterile paddy husk bedding, under standard laboratory conditions: a 12-hour light/dark cycle, ambient temperature of 25 ± 2°C, and relative humidity of 55 ± 5%. They received standard pelletized rodent feed (Vital Feeds®) and clean drinking water ad libitum.

All animals were allowed to acclimatize to the laboratory environment for seven days prior to the commencement of the experiment. All experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Cross River State (Approval Number: UNICROSS/IACUC/2025/012). The study adhered to the ARRIVE guidelines for reporting animal research.

Induction of Diabetes and Experimental Animal Grouping

Diabetes mellitus was induced in fasted rats (12–16 hours) by a single intraperitoneal (i.p.)

injection of freshly prepared streptozotocin (STZ) solution at a dose of 60 mg/kg body weight. STZ was dissolved in cold 0.1 M citrate buffer (pH 4.5) immediately before use and administered within 15 minutes of preparation to prevent degradation. Normal control animals received an equivalent volume of citrate buffer by the same route. At 72 hours post-injection, fasting blood glucose was measured from tail vein blood using a calibrated glucometer (Accu-Chek Performa, Roche, Germany). Rats with fasting blood glucose ≥ 200 mg/dL (≥ 11.1 mmol/L) were confirmed as diabetic and included in the study (Eleazu *et al.*, 2013; ADA, 2023).

Experimental Design

Following confirmation of diabetes, the thirty animals were assigned to five groups (n = 6 per group) as shown in Table 1.

Drug and Extract Administration

All treatments were administered once daily for 28 consecutive days by oral gavage using stainless steel feeding needles (18G). SMESE was freshly prepared each day by dissolving the appropriate amount of extract in distilled water to achieve the desired concentrations. The vehicle for all treatments was distilled water. Metformin was dissolved in distilled water to achieve a concentration of 100 mg/kg body weight. The administration volume was 1 mL/100 g body weight for all treatments, and doses were adjusted weekly based on body weight measurements. Treatment commenced 72 hours after STZ injection, following confirmation of diabetes.

Table 1: Experimental Design

Group	Description	Treatment	N
Group I	Normal control	Distilled water only (oral), no STZ	6
Group II	Diabetic control	STZ-induced; distilled water only (oral)	6
Group III	Low-dose extract	STZ + SMESE 200 mg/kg body weight (oral)	6
Group IV	High-dose extract	STZ + SMESE 400 mg/kg body weight (oral)	6
Group V	Standard drug control	STZ + Metformin 100 mg/kg body weight (oral)	6

SMESE = *Silybum marianum* Ethanol Seed Extract; STZ = Streptozotocin (60 mg/kg i.p., single dose)

Drug and Extract Administration

All treatments were administered once daily for 28 consecutive days by oral gavage using stainless steel feeding needles (18G). SMESE was freshly prepared each day by dissolving the appropriate amount of extract in distilled water to achieve the desired concentrations. The vehicle for all treatments was distilled water. Metformin was dissolved in distilled water to achieve a concentration of 100 mg/kg body weight. The administration volume was 1 mL/100 g body weight for all treatments, and doses were adjusted weekly based on body weight measurements. Treatment commenced 72 hours after STZ injection, following confirmation of diabetes.

Sample Collection and Preparation

Twenty-four hours after the final treatment on Day 28 (i.e., on Day 29), all experimental animals were fasted overnight with unrestricted access to water. The rats were then anaesthetised via intraperitoneal injection of a ketamine/xylazine mixture (75/10 mg/kg body weight). Blood samples of approximately 2–3 mL were collected from each animal via cardiac puncture into plain tubes. The blood was allowed to clot at room temperature for 30 minutes, and then centrifuged at 3000 rpm for 15 minutes using a bench-top centrifuge. The resulting serum was carefully aspirated into labelled Eppendorf tubes and stored at -80°C

pending biochemical analyses. Following blood collection, each rat was euthanised humanely by cervical dislocation under anaesthesia. Animals were dissected via a midline abdominal incision. The kidneys was carefully excised, freed of adhering connective tissue and fat, and rinsed in ice-cold normal saline (0.9% NaCl). Each organ was blotted dry using filter paper and weighed on an analytical balance. One portion of each kidney was homogenised in ice-cold 0.1 M phosphate buffer (pH 7.4) at a ratio of 1:10 (w/v) and centrifuged at 10,000 × g for 15 minutes at 4°C; the resulting supernatant (tissue homogenate) was used for antioxidant enzyme assays. The remaining kidney portion was fixed in 10% neutral-buffered formalin for histopathological processing.

Fasting Blood Glucose Determination

Fasting blood glucose levels were determined using the glucose oxidase-peroxidase (GOD-POD) colourimetric method (Trinder, 1969) using a commercial diagnostic kit (Randox, UK). Absorbance was measured at 505 nm using a UV-Vis spectrophotometer, and glucose concentrations were calculated from a standard calibration curve. Results were expressed in mmol/L.

Serum Insulin Determination

Serum insulin levels were determined using a commercial rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Sweden) according to the manufacturer's instructions. The assay is based on the sandwich ELISA principle using two monoclonal antibodies directed against different epitopes on the insulin molecule. Absorbance was measured at 450 nm using a microplate reader (BioTek ELx800). Insulin concentrations were calculated from a standard curve and expressed in µIU/mL.

Determination of Malondialdehyde (MDA) - Lipid Peroxidation Assay

Lipid peroxidation in kidney tissue homogenates was assessed by measuring thiobarbituric acid reactive substances (TBARS) expressed as MDA equivalents, using the method of Ohkawa *et al*. (1979). Briefly, 0.2 mL of tissue homogenate was mixed with 0.2 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of 20% acetic acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was made up to 4 mL with distilled water and heated at 95°C in a water bath for 60 minutes. After cooling, 1 mL of distilled water and 5 mL of a butanol/pyridine mixture (15:1 v/v) were added and the mixture vortexed vigorously. The organic layer was separated by centrifugation at 4000 rpm for 10 minutes, and its absorbance was measured at 532 nm. MDA concentrations were calculated using 1,1,3,3-tetraethoxypropane as the standard and expressed as nmol/mg protein.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was determined by the nitroblue tetrazolium (NBT) reduction method of Kakkar *et al*. (1984). The assay is based on the inhibition of NBT reduction by the superoxide radical, catalysed by SOD. Briefly, the reaction mixture contained 1.2 mM NBT, 0.1 mM EDTA, 50 mM carbonate buffer (pH 10.2), and 60 µM epinephrine. The reaction was initiated by adding 0.1 mL of tissue homogenate. Absorbance was read at 560 nm at 30-second intervals for 3 minutes. SOD activity was expressed as units/mg protein, where one unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of NBT reduction.

Determination of Catalase (CAT) Activity

Catalase activity was measured by the method of Aebi (1984), based on the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 30 mM H_2O_2. The reaction was initiated by adding 0.1 mL of tissue homogenate. The decrease in absorbance at 240 nm was monitored for 60 seconds. CAT activity was expressed as µmol H_2O_2 decomposed per minute per milligram of protein (µmol/min/mg protein), using the molar extinction coefficient of H_2O_2 (40 M^{-1} cm^{-1}).

Determination of Reduced Glutathione (GSH)

Reduced glutathione levels in kidney tissue were quantified using the method of Ellman (1959), utilising 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). Briefly, 0.5 mL of tissue homogenate was deproteinised with 0.5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 minutes. The supernatant (0.5 mL) was mixed with 1.5 mL of 0.1 M phosphate buffer (pH 7.4) and 0.5 mL of DTNB solution. The yellow colour developed was read

at 412 nm. GSH concentrations were determined from a standard curve and expressed as µmol/mg protein.

Serum Creatinine Determination
Serum creatinine was determined by the alkaline picrate (Jaffe) colourimetric method (Bartels *et al.*, 1972). In the Jaffe reaction, creatinine reacts with picric acid in alkaline solution to form an orange-red coloured Janovsky complex. The intensity of this colour is directly proportional to the creatinine concentration in the sample. Briefly, serum was deproteinised with sodium tungstate and sulfuric acid. The supernatant was mixed with alkaline picrate reagent and the absorbance was read at 520 nm after 20 minutes. Creatinine concentrations were calculated from a standard curve and expressed in mg/dL.

Serum Urea Determination
Serum urea was quantified by the enzymatic urease-Berthelot (indophenol) method using a commercial kit (Randox, UK) (Fawcett & Scott, 1960). Urea in the sample is hydrolysed by urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with sodium salicylate and sodium hypochlorite in the presence of nitroprusside (Berthelot reaction) to form a green-coloured indophenol complex. Absorbance was measured at 600 nm and urea concentrations were calculated from a standard curve. Results were expressed in mg/dL.

Total Protein Determination
Total protein concentration in kidney tissue homogenates was determined by the Bradford (1976) colourimetric method, using bovine serum albumin (BSA) as the standard. The assay is based on the binding of Coomassie Brilliant Blue G-250 dye to protein, causing a shift in absorbance from 465 to 595 nm, the magnitude of which is proportional to protein concentration. Absorbance was read at 595 nm, and protein concentrations were determined from the BSA standard curve. Results were expressed in mg/mL and used as the denominator for specific enzyme activities.

Histopathological Examination
Fixed kidney tissues preserved in 10% neutral-buffered formalin were processed for histopathological evaluation using standard laboratory procedures. Tissues were dehydrated through a graded series of ethanol (70%, 80%, 90%, and 100%), cleared in xylene (two changes), and embedded in paraffin wax using an automated tissue processor (Leica ASP 300 S). Paraffin blocks were sectioned at 4–5 µm thickness using a rotary microtome (Leica RM 2235). The sections were mounted on clean glass slides, deparaffinised in xylene, and rehydrated through descending grades of ethanol before staining with haematoxylin and eosin (H&E).
The H&E-stained sections were examined under a light microscope (Olympus CX43) at magnifications of ×100 and ×400 by a certified veterinary pathologist who was blinded to group assignments. Kidney sections were evaluated for the following pathological features: glomerular integrity, glomerular atrophy, tubular degeneration, interstitial inflammation, and vascular congestion.

Statistical Analysis
All quantitative data were expressed as mean ± standard deviation (SD). Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. One-way analysis of variance (ANOVA) was used to compare experimental groups. When significant differences were found ($p < 0.05$), Tukey's Honest Significant Difference (HSD) post hoc test was used to identify specific pairs of groups that differed significantly. All statistical analyses were performed using IBM SPSS Statistics Version 26.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism Version 9.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$ for all comparisons.

Results

This chapter presents the results of the study systematically, encompassing: (i) the percentage yield and phytochemical composition of the extract; (ii) the acute oral toxicity profile (LD50); (iii) effects on fasting blood glucose and serum insulin; (iv) renal function markers; (v) antioxidant enzyme activities in kidney tissue; (vi) total protein levels; and (vii) histopathological findings in kidney tissues.

Percentage Yield and Phytochemical Screening	The ethanol extraction of *Silybum marianum* seeds from 500 g of powdered material yielded 42.6 g of dry extract, corresponding to a percentage yield of 8.52% (w/w).

Table 2: Quantitative Yield and Semi-Quantitative Phytochemical Profile of SMESE

Phytochemical	Presence	Dry Weight	Powdered Material Yielded	Percentage Yield
Flavonoids	+++			
Tannins	++			
Saponins	+	500 g	42.6 g of dry extract	8.52%
Terpenoids	++			
Alkaloids	+			
Phenolic compounds	+++			
Steroids	+			
Cardiac glycosides	-			

Key: +++ = highly abundant; ++ = moderately abundant; + = present in low concentration; - = absent

The phytochemical screening revealed that flavonoids and phenolic compounds were highly abundant (+++), tannins and terpenoids were moderately abundant (++), while saponins, alkaloids, and steroids were present in lower concentrations (+). Cardiac glycosides were completely absent (-).

Table 3: Acute Oral Toxicity of SMESE (Lorke's Method)

Phase	Dose (mg/kg)	Mortality	Observation
I	10	0/3	No signs of toxicity
I	100	0/3	No signs of toxicity
I	1000	0/3	No signs of toxicity
II	1600	0/3	No signs of toxicity
II	2900	0/3	No signs of toxicity
II	5000	1/3	Death on Day 3

3.2 Acute Oral Toxicity (LD50)

In the Lorke (1983) Phase I study, no mortality was recorded in any of the three groups administered SMESE at 10, 100, or 1000 mg/kg body weight over the 14-day observation period. Based on these Phase I results, Phase II doses of 1600, 2900, and 5000 mg/kg were tested. No mortality occurred at 1600 or 2900 mg/kg; however, one of the three animals dosed at 5000 mg/kg died by Day 3.

Since 100% mortality was not achieved at any tested dose, the exact LD50 could not be calculated using the Lorke formula. The LD50 of SMESE is therefore reported as >2900 mg/kg body weight, classifying it as practically non-toxic (GHS Category 5). The experimental doses selected were 200 mg/kg and 400 mg/kg, which represent approximately 1/15 and 1/7 of the highest non-lethal dose tested, respectively, thereby ensuring a wide margin of safety for experimental animals throughout the study.

Table 4: Effect of SMESE on Fasting Blood Glucose Levels (mmol/L)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Group I -Normal control	4.6 ± 0.3	4.7 ± 0.4	4.8 ± 0.3	4.8 ± 0.4	4.8 ± 0.4
Group II -Diabetic control	17.8 ± 1.5[a]	18.2 ± 1.6[a]	18.9 ± 1.7[a]	19.1 ± 1.8[a]	19.4 ± 1.9[a]
Group III - SMESE 200 mg/kg	17.5 ± 1.3	15.2 ± 1.2*	13.4 ± 1.1*	11.8 ± 1.0*	10.5 ± 1.0*
Group IV -SMESE 400 mg/kg	17.6 ± 1.4	14.1 ± 1.1*	11.8 ± 0.9*	9.6 ± 0.8*	8.1 ± 0.9*
Group V-Metformin 100 mg/kg	17.4 ± 1.3	13.8 ± 1.0*	11.2 ± 0.8*	9.1 ± 0.7*	7.4 ± 0.7*

Values are expressed as mean ± SD (n=6). [a]Significantly different from normal control (Group I) at $p < 0.05$. *Significantly different from diabetic control (Group II) at $p < 0.05$ (one-way ANOVA; Tukey's HSD post hoc test).

The fasting blood glucose levels in the normal control group remained stable throughout the experimental period (mean: 4.8 ± 0.4 mmol/L on Day 28). In contrast, the diabetic control group exhibited markedly elevated fasting blood glucose from Day 0 (post-STZ induction) that remained consistently high throughout the study period (19.4 ± 1.9 mmol/L on Day 28). SMESE treatment produced dose-dependent reductions in fasting blood glucose. The high-dose group (400 mg/kg) demonstrated a progressive reduction from 17.6 ± 1.4 mmol/L at baseline to 8.1 ± 0.9 mmol/L at Day 28, approaching the metformin group value of 7.4 ± 0.7 mmol/L.

Table 5: Effect of SMESE on Serum Insulin Levels

Group	Serum Insulin (µIU/mL)
Group I - Normal control	15.2 ± 1.3
Group II- Diabetic control	5.8 ± 0.9[a]
Group III- SMESE 200 mg/kg	9.6 ± 1.1*
Group IV- SMESE 400 mg/kg	13.1 ± 1.4*
Group V- Metformin 100 mg/kg	14.0 ± 1.2*

Values are expressed as mean ± SD (n=6). [a]Significantly different from normal control (Group I) at $p < 0.05$. *Significantly different from diabetic control (Group II) at $p < 0.05$ (one-way ANOVA; Tukey's HSD post hoc test).

Treatment with SMESE produced a dose-dependent restoration of serum insulin, with the high-dose group (400 mg/kg) achieving 13.1 ± 1.4 µIU/mL, closely approaching the metformin group (14.0 ± 1.2 µIU/mL).

Table 6: Effect of SMESE on Serum Creatinine Levels

Serum creatinine levels were significantly elevated in the diabetic control group (1.45 ± 0.12 mg/dL) compared to the normal control (0.72 ± 0.08 mg/dL). SMESE treatment produced dose-dependent

Group	Serum Creatinine (mg/dL)
Group I- Normal control	0.72 ± 0.08
Group II -Diabetic control	1.45 ± 0.12[a]
Group III -SMESE 200 mg/kg	1.12 ± 0.10*
Group IV -SMESE 400 mg/kg	0.85 ± 0.09*
Group V-Metformin 100 mg/kg	0.80 ± 0.07*

Values are expressed as mean ± SD (n=6). [a]Significantly different from normal control (Group I) at $p < 0.05$. *Significantly different from diabetic control (Group II) at $p < 0.05$ (one-way ANOVA; Tukey's HSD post hoc test).

Table 7: Effect of SMESE on Serum Urea and Total Protein Levels

Group	Urea (mg/dL)	Total Protein (mg/mL)
Group I -Normal control	18.4 ± 1.6	7.2 ± 0.5
Group II -Diabetic control	48.6 ± 3.2[a]	4.8 ± 0.4[a]
Group III -SMESE 200 mg/kg	32.5 ± 2.8*	5.6 ± 0.5*
Group IV -SMESE 400 mg/kg	22.8 ± 2.1*	6.7 ± 0.5*
Group V- Metformin 100 mg/kg	20.5 ± 1.8*	6.9 ± 0.4*

Serum urea levels were significantly elevated in the diabetic control group (48.6 ± 3.2 mg/dL) compared to the normal control (18.4 ± 1.6 mg/dL). Treatment with SMESE produced dose-dependent reductions in serum urea: the low-dose group recorded 32.5 ± 2.8 mg/dL, while the high-dose group recorded 22.8 ± 2.1 mg/dL, approaching the metformin group value of 20.5 ± 1.8 mg/dL. Total protein concentrations were significantly reduced in the diabetic control group (4.8 ± 0.4 mg/mL) compared to the normal control (7.2 ± 0.5 mg/mL). SMESE administration restored total protein levels in a dose-dependent manner, with the high-dose group recording 6.7 ± 0.5 mg/mL, comparable to the metformin group (6.9 ± 0.4 mg/mL).

Values are expressed as mean ± SD (n=6). [a]Significantly different from normal control (Group I) at $p < 0.05$. *Significantly different from diabetic control (Group II) at $p < 0.05$ (one-way ANOVA; Tukey's HSD post hoc test).

The STZ-induced diabetic control group exhibited markedly depressed antioxidant enzyme activities compared to the normal control group. Specifically, SOD activity was significantly reduced from 28.6 ± 2.1 U/mg protein (normal control) to 11.3 ± 1.4 U/mg protein (diabetic control; $p < 0.05$). CAT activity declined from 18.4 ± 1.6 µmol H_2O_2/min/mg protein to 7.2 ± 0.9 µmol H_2O_2/min/mg protein. GSH levels were reduced from 42.3 ± 3.2 µmol/mg protein to 18.6 ± 2.1 µmol/mg protein. MDA levels were significantly elevated from 2.8 ± 0.4 nmol/mg protein in the normal control to 9.6 ± 0.8 nmol/mg protein in the diabetic control, indicating profound oxidative stress in the diabetic kidney. SMESE administration resulted in dose-dependent restoration of antioxidant enzyme activities and suppression of lipid peroxidation. In the high-dose group (400 mg/kg), SOD activity was restored to 23.4 ± 1.8 U/mg protein, CAT activity to 14.8 ± 1.3 µmol H_2O_2/min/mg protein, and GSH to 35.7 ± 2.6 µmol/mg protein, while MDA was

significantly reduced to 4.2 ± 0.5 nmol/mg protein. These values approached those of the metformin group (SOD: 24.1 ± 1.9; CAT: 15.2 ± 1.4; GSH: 36.9 ± 2.8; MDA: 3.9 ± 0.4) and were significantly different from the diabetic control ($p < 0.05$).

Table 8: Effect of SMESE on Renal Antioxidant Enzyme Activities and Lipid Peroxidation

Group	SOD (U/mg protein)	CAT (µmol H_2O_2/min/mg protein)	GSH (µmol/mg protein)	MDA (nmol/mg protein)
Group I -Normal control	28.6 ± 2.1	18.4 ± 1.6	42.3 ± 3.2	2.8 ± 0.4
Group II-Diabetic control	11.3 ± 1.4[a]	7.2 ± 0.9[a]	18.6 ± 2.1[a]	9.6 ± 0.8[a]
Group III- SMESE 200 mg/kg	17.8 ± 1.6*	10.9 ± 1.1*	26.4 ± 2.3*	6.8 ± 0.6*
Group IV- SMESE 400 mg/kg	23.4 ± 1.8*	14.8 ± 1.3*	35.7 ± 2.6*	4.2 ± 0.5*
Group V- Metformin 100 mg/kg	24.1 ± 1.9*	15.2 ± 1.4*	36.9 ± 2.8*	3.9 ± 0.4*

Values are expressed as mean ± SD (n=6). [a]Significantly different from normal control (Group I) at $p < 0.05$. *Significantly different from diabetic control (Group II) at $p < 0.05$ (one-way ANOVA; Tukey's HSD post hoc test). SOD = Superoxide Dismutase; CAT = Catalase; GSH = Reduced Glutathione; MDA = Malondialdehyde.

The diabetic control group demonstrated the highest kidney damage score (3.8 ± 0.3), reflecting severe glomerular atrophy, tubular degeneration, interstitial inflammation, and vascular congestion observed on H&E-stained sections.

Figure 1: Photomicrographs of Kidney Tissues (H&E staining, ×400)

(A) Normal control group showing intact glomerular tufts, patent Bowman's spaces, and well-organised tubular epithelium. (B) Diabetic control group showing severe glomerular atrophy with collapsed capillary tufts, dilated Bowman's spaces, extensive tubular degeneration with loss of brush border, and moderate interstitial inflammatory cell infiltration. (C) Low-dose SMESE (200 mg/kg) group showing partial recovery with discernible glomerular profiles and reduced tubular vacuolation. (D) High-dose SMESE (400 mg/kg) group demonstrating near-normal renal architecture with intact glomeruli, well-formed tubules, and minimal interstitial changes. (E) Metformin-treated group showing significant restoration of normal renal histological architecture.

Table 9: Histopathological Scores for Kidney Damage Integrity

Group	Kidney Damage Score (0–4)
Group I - Normal control	0.5 ± 0.2
Group II - Diabetic control	3.8 ± 0.3[a]
Group III -SMESE 200 mg/kg	2.5 ± 0.4*
Group IV - SMESE 400 mg/kg	1.2 ± 0.3*
Group V - Metformin 100 mg/kg	1.0 ± 0.2*

Values are expressed as mean ± SD (n=6). [a]Significantly different from normal control (Group I) at $p < 0.05$. *Significantly different from diabetic control (Group II) at $p < 0.05$ (one-way ANOVA; Tukey's HSD post hoc test). Score interpretation: Kidney damage: 0 = normal architecture, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe damage.

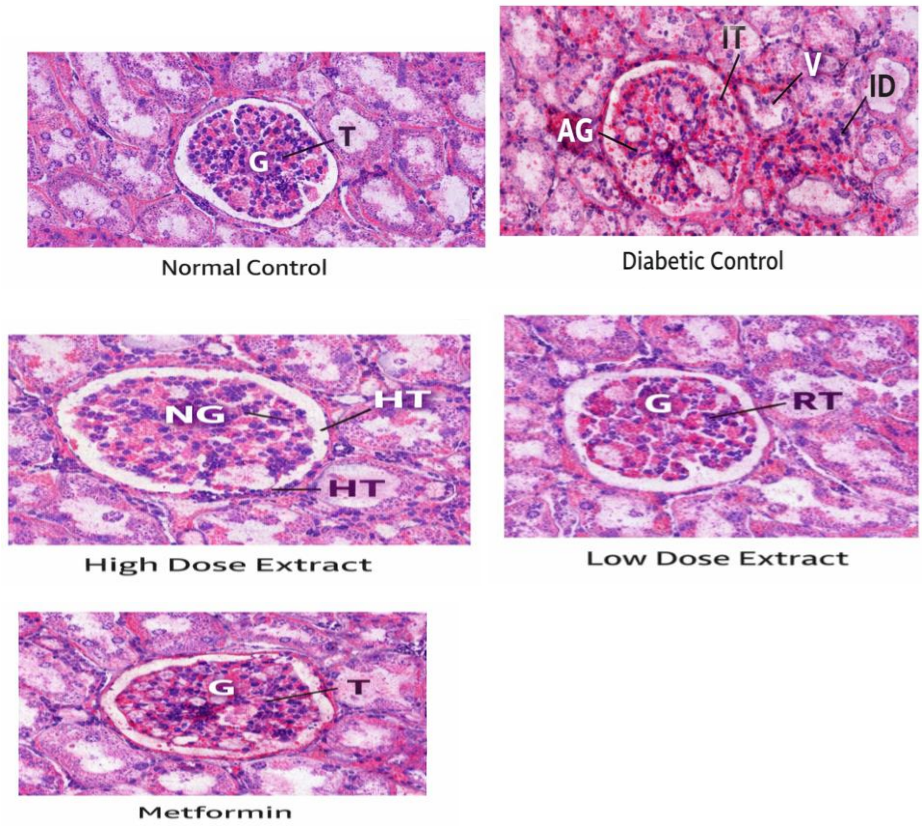

Figure 1 Photomicrographic Description of histopathology of the kidney

Discussion

The present study investigated the antihyperglycaemic, antioxidant, and nephroprotective effects of the ethanol seed extract of *Silybum marianum* (SMESE) in a streptozotocin-induced diabetic rat model. The findings collectively demonstrate that SMESE exerts significant, dose-dependent therapeutic effects across all evaluated outcome domains, with the high-dose group (400 mg/kg) consistently yielding results that approached those of the standard reference drug, metformin (100 mg/kg). The 8.52% extraction yield indicates efficient recoverability of active components. This yield is comparable to values reported by Maaliah *et al.* (2024) for similar extraction protocols. The phytochemical screening revealed a rich and diverse array of secondary metabolites, with a distinct dominance of polyphenolic compounds (flavonoids and phenolics) (Njar *et al*., 2026). This profile strongly suggests that the plant extract is likely to exhibit pronounced antioxidant, anti-inflammatory, and broad-spectrum antimicrobial activities. The strong positive reactions for flavonoids and phenolics are consistent with the established phytochemical profile of *Silybum marianum*, whose pharmacological activity is primarily attributable to its flavonolignan complex (silymarin) (Abenavoli *et al*., 2018; Gazak *et al*., 2007). The conspicuous absence of cardiac glycosides enhances the safety margin of this extract for future in vivo studies. Cardiac glycosides are known to inhibit the Na^+/K^+-ATPase pump and can cause severe cardiotoxicity. Their complete absence significantly reduces a major risk factor associated with the use of this plant extract.

The LD50 of SMESE was determined to be greater than 2900 mg/kg body weight, as no mortality occurred at this dose and only one of three animals died at 5000 mg/kg. This value classifies SMESE as practically non-toxic under the Globally Harmonised System (GHS) of chemical classification (Category 5), consistent with the widely reported favourable safety profile of *Silybum marianum* in the published literature (Abenavoli *et al*., 2018). The selected experimental doses of 200 mg/kg and 400 mg/kg, representing approximately 1/15 and 1/7 of the highest non-lethal dose tested, respectively, therefore provided a satisfactory margin of safety for all experimental animals. These findings are consistent with earlier reports indicating that the LD50 of silymarin and silibinin is greater than 2000 mg/kg (Szkudelski, 2001; Lenzen, 2008; Awla *et al*., 2023).

Treatment with SMESE produced a dose-dependent restoration of serum insulin, with the high-dose group (400 mg/kg) achieving 13.1 ± 1.4 µIU/mL — closely approaching the metformin group (14.0 ± 1.2 µIU/mL). The mechanism likely involves the antioxidant-mediated preservation of residual beta-cell mass, reduction of apoptosis, and improved beta-cell sensitivity to glucose stimulation. The progressive, dose-dependent reduction in fasting blood glucose observed in SMESE-treated groups, from 17.6 ± 1.4 mmol/L at baseline to 8.1 ± 0.9 mmol/L at Day 28 in the high-dose group, demonstrates the antihyperglycaemic activity of the extract. These findings are consistent with those of Maaliah *et al*. (2024), who reported that SMESE administration significantly decreased serum glucose levels in STZ-induced diabetic rats. The antihyperglycaemic effect of SMESE may be attributed to multiple mechanisms, including inhibition of α-amylase activity, enhancement of insulin secretion, and improvement of peripheral insulin sensitivity (Maaliah *et al*., 2024).

The significantly depressed SOD, CAT, and GSH activities and elevated MDA levels observed in the diabetic control group reflect the profound oxidative stress associated with chronic hyperglycaemia, a consequence of excessive ROS generation from multiple metabolic pathways including glucose autooxidation, mitochondrial electron transport chain dysfunction, and NADPH oxidase activation (Giacco & Brownlee, 2010; Brownlee, 2001). Elevated MDA, a principal end-product of lipid peroxidation, indicates extensive membrane damage in diabetic renal tissue (Esterbauer *et al*., 1991). SMESE administration produced a dose-dependent restoration of antioxidant enzyme activities and suppression of lipid peroxidation. The high-dose group demonstrated near-complete restoration of SOD, CAT, and GSH activities to levels approaching those of the normal control and metformin groups. These findings are consistent with prior studies demonstrating that

silymarin upregulates the expression of SOD, CAT, and GPx through activation of the Nrf2/ARE signalling pathway — a master regulator of cellular antioxidant defence (Gazak *et al*., 2007; Bai *et al*., 2016). The study by Awla *et al*. (2023), employing LC-MS/MS profiling and comprehensive antioxidant characterisation of *Silybum marianum* metabolites, further corroborates the potent free-radical scavenging capacity of the plant's phytochemical constituents.

The reduction in MDA levels from 9.6 ± 0.8 nmol/mg protein in the diabetic control to 4.2 ± 0.5 nmol/mg protein in the high-dose SMESE group indicates significant protection against lipid peroxidation in renal tissue. This is consistent with the findings of Vessal *et al*. (2010), who demonstrated that milk thistle extract attenuates diabetic renal damage by decreasing lipid peroxidation in renal tissue and increasing kidney CAT and GPx activity.

The significant elevation of serum creatinine (1.45 ± 0.12 mg/dL) and serum urea (48.6 ± 3.2 mg/dL) in the diabetic control group compared to the normal control (creatinine: 0.72 ± 0.08 mg/dL; urea: 18.4 ± 1.6 mg/dL) is a classical indicator of reduced glomerular filtration rate (GFR), consistent with STZ-induced diabetic nephropathy. Creatinine, freely filtered by the glomerulus and neither substantially reabsorbed nor secreted in the healthy kidney, accumulates in the circulation when nephron function is compromised (Levey *et al*., 2003).

The progressive reduction in serum creatinine to 0.85 ± 0.09 mg/dL and serum urea to 22.8 ± 2.1 mg/dL following high-dose SMESE administration indicates restoration of glomerular filtration capacity. These findings align closely with those reported by Sheela *et al*. (2013), who reported significant reductions in serum creatinine, blood urea nitrogen, and glycosylated haemoglobin following silymarin administration in STZ-nicotinamide-induced diabetic nephropathic rats. The restoration of total protein levels from 4.8 ± 0.4 mg/mL (diabetic control) to 6.7 ± 0.5 mg/mL (high-dose SMESE) indicates preservation of the glomerular filtration barrier integrity and reduced urinary protein loss. This is consistent with the histopathological findings of preserved glomerular architecture in extract-treated groups. The underlying mechanism of nephroprotection likely involves multiple interrelated pathways: (i) suppression of ROS-mediated podocyte injury through antioxidant activity; (ii) inhibition of NF-κB-driven proinflammatory cytokine production; (iii) attenuation of TGF-β1-mediated mesangial fibrosis; and (iv) upregulation of Nrf2/HO-1 cytoprotective signalling (Navarro-González *et al*., 2011; Yaribeygi *et al*., 2020; Bai *et al*., 2016). These mechanisms are proposed based on established literature but were not directly measured in this study and should be considered speculative.

The histopathological findings of this study corroborate and extend the biochemical evidence. The severe renal damage observed in the diabetic control group — characterised by glomerular atrophy, collapse of capillary tufts, tubular vacuolation, and interstitial inflammatory infiltration is consistent with the well-documented histopathology of STZ-induced diabetic nephropathy (Fioretto & Mauer, 2007). The high-dose SMESE group (400 mg/kg) consistently produced outcomes approaching those of the metformin-treated group (100 mg/kg) across all evaluated parameters: fasting blood glucose (8.1 ± 0.9 vs. 7.4 ± 0.7 mmol/L), serum insulin (13.1 ± 1.4 vs. 14.0 ± 1.2 µIU/mL), serum creatinine (0.85 ± 0.09 vs. 0.80 ± 0.07 mg/dL), serum urea (22.8 ± 2.1 vs. 20.5 ± 1.8 mg/dL), and antioxidant enzyme activities. These findings suggest that SMESE at 400 mg/kg possesses antihyperglycaemic and nephroprotective efficacy approaching that of metformin, the first-line pharmacological agent for T2DM (ADA, 2023).

This is particularly significant given the limitations of metformin, including gastrointestinal side effects and contraindication in patients with renal impairment due to the risk of lactic acidosis. The favourable safety profile of SMESE (LD50 >2900 mg/kg) suggests that it may offer a safer alternative or adjunctive therapy for diabetic patients, particularly in resource-constrained settings where access to conventional pharmaceuticals may be limited.

Study Limitations

This study has several limitations that should be acknowledged:The STZ-induced diabetes model produces insulin-deficient, type 1-like diabetes. While metformin was used as a reference drug due to its established antioxidant and nephroprotective properties, the findings may not fully translate to type 2 diabetes pathophysiology. The 28-day study period

limits assessment of long-term efficacy and chronic toxicity. Molecular mechanisms (Nrf2/ARE, NF-κB, TGF-β1 signalling) were not directly measured and are proposed based on literature. Individual bioactive compounds were not isolated or quantified using HPLC/LC-MS/MS. The absorption, distribution, metabolism, and excretion profile of SMESE was not characterized.

Conclusion

The findings of this study demonstrate that the ethanol seed extract of *Silybum marianum* possesses significant antihyperglycaemic, antioxidant, and nephroprotective properties in streptozotocin-induced diabetic albino rats. The extract, at both doses evaluated, produced dose-dependent improvements in fasting blood glucose, serum insulin levels, antioxidant enzyme activities (SOD, CAT, GSH), lipid peroxidation markers (MDA), renal function biomarkers (serum creatinine, serum urea, total protein), and histopathological architecture of kidney tissues. The high dose of 400 mg/kg consistently produced outcomes approaching those of the reference drug, metformin (100 mg/kg). The LD50 of greater than 2900 mg/kg body weight confirms the safety of the extract at the doses employed. These findings provide experimental evidence that *Silybum marianum* may represent a promising plant-derived adjunctive therapeutic option for the management of diabetic nephropathy. The study contributes to the growing scientific evidence base for the validation of traditional medicinal plant use. However, clinical translation requires further mechanistic studies, chronic toxicity evaluation, pharmacokinetic characterisation, and ultimately human clinical trials before therapeutic claims can be made.

Recommendations

Considering the findings of this study, the following recommendations are proposed: Future studies should extend the evaluation period beyond 28 days to determine whether the nephroprotective and antihyperglycaemic effects of SMESE are sustained with prolonged administration and to assess potential chronic toxicity. Pharmacokinetic studies are recommended to characterise the absorption, distribution, metabolism, and excretion (ADME) profile of SMESE, particularly the bioavailability of the silymarin complex following oral administration. Isolation and identification of individual bioactive constituents of SMESE using HPLC, LC-MS/MS, or GC-MS techniques is recommended, alongside pharmacological evaluation of individual constituents. Mechanistic studies employing Western blotting, quantitative real-time PCR (qRT-PCR), and immunohistochemistry are recommended to elucidate the molecular targets of SMESE, particularly regarding Nrf2/ARE pathway activation, NF-κB inhibition, and TGF-β1 modulation. Evaluation of SMESE in a type 2 diabetic model (e.g., high-fat diet combined with low-dose STZ) is recommended to broaden the translational applicability of these findings. Combination therapy studies examining the efficacy and safety of SMESE in combination with standard antidiabetic agents (metformin, glibenclamide) are warranted. Clinical translation through pilot randomised controlled trials in human subjects with diabetic nephropathy is recommended as the ultimate step in validating the therapeutic utility of *Silybum marianum*.

Declarations

Author Contributions: All authors contributed equally.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: All experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Cross River State (Approval Number: UNICROSS/IACUC/2025/012).

Funding: Not applicable

AI Disclosure: This article was researched and drafted with the assistance of artificial intelligence. While AI was used to generate initial concepts and structure, the final content has been thoroughly reviewed, fact-checked, and edited by a human author to ensure accuracy, originality, and adherence to our editorial standards.

Data Availability: The data supporting the findings are contained in this article of this

study and are available from the corresponding author upon reasonable request.

References

Abenavoli, L., Izzo, A. A., Milic, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (*Silybum marianum*): A concise overview of its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytotherapy Research*, 32(11), 2202–2213.

Abu-Alkaseem, B. A., Hamzah, M. I., & Raghif, A. R. A. (2018). Effect of ethyl acetate fraction of *Silybum marianum* and *Ficus carica* leaves extract on biochemical parameters and histopathological outcomes in streptozotocin-induced T2DM rats. *Journal of Pharmaceutical Sciences and Research*, 10(3), 547–552.

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121–126.

Akinluge, O. O. (2003). *Non-communicable diseases in Nigeria: National survey final report*. Federal Ministry of Health, Lagos.

American Diabetes Association (ADA). (2023). Standards of care in diabetes—2023. *Diabetes Care*, 46(Suppl. 1), S1–S291.

Awla, N. J., Naqishbandi, A. M., & Baqi, Y. (2023). Preventive and therapeutic effects of *Silybum marianum* seed extract rich in silydianin and silychristin in a rat model of metabolic syndrome. *ACS Pharmacology & Translational Science*, 6(11), 1613–1625.

Bai, T., Yang, Y., Wu, Y.-L., Jiang, S., Lee, J.-J., & Nan, J.-X. (2016). Silibinin alleviates high glucose-induced fibrosis in mouse glomerular mesangial cells by inhibiting epithelial-to-mesenchymal transition. *Cell Biology International*, 36(9), 857–863.

Bartels, H., Böhmer, M., & Heierli, C. (1972). Serum creatinine determination without protein precipitation. *Clinica Chimica Acta*, 37, 193–197.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414(6865), 813–820.

Eleazu, C. O., Eleazu, K. C., Chukwuma, S., & Essien, U. N. (2013). Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its utility in the development of a model for type 2 diabetes. *Journal of Diabetes and Metabolic Disorders*, 12(1), 60.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1), 70–77.

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*, 11(1), 81–128.

Fawcett, J. K., & Scott, J. E. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*, 13(2), 156–159.

Fioretto, P., & Mauer, M. (2007). Histopathology of diabetic nephropathy. *Seminars in Nephrology*, 27(2), 195–207.

Forbes, J. M., Coughlan, M. T., & Cooper, M. E. (2008). Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes*, 57(6), 1446–1454.

Gazak, R., Walterova, D., & Kren, V. (2007). Silybin and silymarin—New and emerging applications in medicine. *Current Medicinal Chemistry*, 14(3), 315–338.

Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research*, 107(9), 1058–1070.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman and Hall.

Huseini, H. F., Larijani, B., Heshmat, R., Fakhzadeh, H., Radjabipour, B., Toliat, T., & Raza, M. (2006). The efficacy of *Silybum marianum* (L.) Gaertn. (silymarin) in the treatment of type 2 diabetes: A randomised, double-blind, placebo-controlled, clinical trial.

Phytotherapy Research, 20(12), 1036–1039.

International Diabetes Federation (IDF). (2021). *IDF Diabetes Atlas* (10th ed.). International Diabetes Federation.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, 21(2), 130–132.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, 51(2), 216–226.

Levey, A. S., Coresh, J., Balk, E., Kausz, A. T., Levin, A., Steffes, M. W., Hogg, R. J., Perrone, R. D., Lau, J., & Eknoyan, G. (2003). National Kidney Foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Annals of Internal Medicine*, 139(2), 137–147.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4), 275–287.

Maaliah, M. S., Haddadin, M., & Abdalla, S. (2024). Hypolipidemic and hypoglycemic effects of *Silybum marianum* (L.) Gaertn. (milk thistle) ethanol seed extract in streptozotocin-induced diabetes in rats. *Journal of Pharmacology and Pharmacotherapeutics*, 20(3), 841–852.

MacDonald-Ramos, K., Michán, L., Martínez-Ibarra, A., & Cerbón, M. (2021). Silymarin is an ally against insulin resistance: A review. *Annals of Hepatology*, 23, 100255.

Navarro-González, J. F., Mora-Fernández, C., Muros de Fuentes, M., Chahin, J., Méndez, M. L., Gallego, E., Macía, M., del Castillo, N., Rivero, A., Getino, M. A., García, P., Jarque, A., & García, J. (2011). Effect of silymarin on kidney function in patients with diabetic nephropathy. *Journal of Diabetes and its Complications*, 25(3), 166–171.

Njar, P.A., Okpashi, V.E., Bayim, P-R.B., Tabe, N.N., Okputu, J.G., & Mbom, M.M (2026). Anti-androgenic and Anti-inflammatory Effects of *Corchorus Olitorius* Leaf Extract in Testosterone-Induced Benign Prostatic Hyperplasia: Biochemical and GC-MS Analysis. *Toxicology Digest 5(1): 57-67. DOI: https://doi.org/10.71637/toxicologydigest.vol5no1.52*

Obi, B. C., Okoye, T. C., Okpashi, V. E., Igwe, C. N., & Alumanah, E. O. (2016). Comparative study of the antioxidant effects of metformin, glibenclamide, and repaglinide in alloxan-induced diabetic rats. *Journal of Diabetes Research*, Article ID 1635361.

Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., Cavan, D., Shaw, J. E., & Makaroff, L. E. (2017). Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice*, 128, 40–50.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358.

Okpashi, V. E., Bayim, P. R. B., & Obi-Abang, M. (2014). Comparative effects of some medicinal plants: *Anacardium occidentale*, *Eucalyptus globulus*, *Psidium guajava* and *Xylopia aethiopica* extracts in alloxan-induced diabetic male Wistar albino rats. *Biochemistry Research International*, Article ID 203051.

Sheela, N., Jose, M. A., Sathyamurthy, D., & Kumar, B. N. (2013). Effect of silymarin on streptozotocin-nicotinamide-induced type 2 diabetic nephropathy in rats. *Iranian Journal of Kidney Diseases*, 7(2), 117–123.

Soto, C., Mena, R., Luna, J., Cerbón, M., Larrieta, E., Vital, P., Uría, E., Soto, L., Recoba, R., Barrón, H., Favari, L., & Lara, A. (2010). Silymarin induces recovery of pancreatic function after alloxan damage in rats. *Life Sciences*, 75(18), 2167–2180.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, 50(6), 537–546.

Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Saunders.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an

alternative oxygen acceptor. *Annals of Clinical Biochemistry*, 6(1), 24–27.

Umanath, K., & Lewis, J. B. (2018). Update on diabetic nephropathy: Core curriculum 2018. *American Journal of Kidney Diseases*, 71(6), 884–895.

Vessal, G., Akmali, M., Najafi, P., Moein, M. R., & Sagheb, M. M. (2010). Silymarin and milk thistle extract may prevent the progression of diabetic nephropathy in streptozotocin-induced diabetic rats. *Renal Failure*, 32(6), 733–739.

Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative Medicine and Cellular Longevity*, 2020, 8609213.