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Antihyperglicemic and Nephroprotective Effects of Freeze-Dried Strawberry (*Fragaria X Ananassa*) in Alloxan-Induced Diabetic Rat

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ABSTRACT

Strawberries (*Fragaria* × *ananassa*) are rich in bioactive compounds and essential vitamins, including flavonoids, phenolic acids, tannins, ascorbic acid, and folate which exhibit antioxidant and anti-hyperglycemic properties. However, evidence on the efficacy of freeze-dried strawberries (FDS) in modulating kidney function and histopathology remains limited. This study investigated the effects of FDS on Fasting Blood Glucose (FBG), albumin-to-creatinine ratio (ACR), and renal histopathology in alloxan-induced diabetic rats. An experimental post-test only control group design was used with 25 Wistar rats (8–12 weeks old) randomly assigned to five groups (n = 5): normal control (K−), untreated diabetic (K+) receiving alloxan injection only, and three diabetic groups treated with alloxan followed by FDS administration at doses of 1000 mg/kg BW (P1), 1500 mg/kg BW (P2), or 2000 mg/kg BW (P3) via orogastric tube for four weeks. Diabetic model was obtained using a single intraperitoneal injection of alloxan at a dosage of 150 mg/kg body weight. FBG, ACR, and renal histopathology (Renal Injury Scoring System) were evaluated at the conclusion of the study. Data were analyzed using one-way ANOVA and the Kruskal–Wallis test. FDS at 2000 mg/kg markedly decreased FBG in comparison to K+ ($p<0.05$). ACR was markedly reduced at dosages of 1000, 1500, and 2000 mg/kg in comparison to K+ ($p<0.05$). Renal histopathological improvements were observed at 1000 1500 and 2000 mg/kg compared with K+ ($p<0.05$). These findings suggest that FDS supplementation exerts dose-dependent antihyperglycaemic and nephroprotective effects in diabetic rats, with different doses favouring specific outcomes.

Keywords: *Fragaria* × *ananassa*, Freeze-dried, Diabetes Mellitus, Fasting Blood Glucose, Diabetic Nephropathy.

Introduction

Diabetes mellitus (DM) represents a growing global health burden associated with a markedly increased risk of premature mortality. According to the International Diabetes Federation an estimated 589 million adults, equivalent to 11.1% of the global population aged 20–79 years, were living with diabetes in 2025, a figure projected to rise to 852 million (13% of the adult population) by 2050.[1] Inadequate glycaemic control contributes to the development of serious complications, including retinopathy, neuropathy, nephropathy, stroke, and cardiovascular disease, all of which substantially increase morbidity and mortality.[2]

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Diabetes accounts for approximately 460,000 deaths annually from renal disease, while hyperglycaemia contributes to around 20% of cardiovascular-related mortality worldwide.[3]

Diabetic nephropathy (DN) is one of the most prevalent and severe complications of diabetes mellitus and remains the leading cause of end-stage renal disease (ESRD) worldwide.[4] It is a multifactorial condition involving the interplay of inflammatory, metabolic, and haemodynamic pathways,[5] as well as genetic and environmental influences.[6] Among these, oxidative stress plays a pivotal role in disease progression.[7] Persistent hyperglycaemia induces excessive production of reactive oxygen species (ROS), leading to oxidative damage that promotes albuminuria, renal inflammation, fibrosis, and a gradual decline in renal function.[8,9]

The potential of plant-based interventions and natural supplements in the management of diabetes has gained increasing recognition alongside advances in conventional pharmacological therapies. There is growing interest in complementary dietary strategies that can safely improve glycaemic control and provide renal protection, as long-term pharmacotherapy is often associated with adverse side effects. Strawberry (*Fragaria* × *ananassa*), a non-climacteric fruit that does not continue to ripen after harvest, belongs to the Rosaceae family and is cultivated worldwide for its distinctive flavour, aroma, and vibrant red colour.[10] Originating from hybridization events between species native to North and South America, it is now extensively cultivated in temperate regions worldwide. The species has also been successfully grown in tropical and subtropical regions under controlled environments or at higher altitudes, such as in Indonesia.[11]

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Strawberry (*Fragaria* × *ananassa*) fruit is valued not only for its sensory qualities but also for its nutritional profile, offering a balanced composition that supports a healthy diet. Importantly, strawberries are rich in bioactive compounds, particularly antioxidants such as vitamin C and polyphenols, including anthocyanins and phenolic acids. These phytochemicals exhibit diverse health-promoting properties, with polyphenols known to play a preventive role in chronic diseases such as cancer, cardiovascular disorders, arthritis, and type 2 diabetes.[10] Moreover, strawberries demonstrate antioxidant, antihyperglycemic, and antihyperlipidaemic effects, reducing oxidative stress and enhancing insulin sensitivity, thereby underscoring their potential as a functional food for diabetes management.[12–14] However, as fresh strawberries are highly perishable, freeze-drying has been shown to be a superior preservation method, effectively maintaining their phytochemical integrity, including tannins, flavonoids, and polyphenols.[15]

This study aims to assess the impact of freeze-dried strawberry (FDS) supplementation on blood glucose levels, the albumin–creatinine ratio (ACR), and renal histopathology in diabetic rat model. The findings are expected to provide scientific evidence supporting the potential of freeze-dried strawberries as a natural dietary intervention for glycaemic regulation and renal protection in diabetes management.

Materials and Methods

Study Design

An *in vivo* experimental study using a posttest-only control group design was conducted to evaluate the effects of FDS supplementation on FBG, ACR, and renal histopathology. The experiment was conducted at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya, between August and September 2024. All methods used in experiments adhered to the guidelines set forth by the Institutional Animal Care and Use Committee (IACUC) and obtained ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya (No. 0401/EC/KEPK/UNUSA/2024).

Animals

The study used male Rattus norvegicus Wistar rats aged 8-12 weeks and weighed 150-200 g, all of whom demonstrated typical activity. The animals were procured from a registered breeding facility in Batu, Malang, and housed in the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Airlangga University in Surabaya. Rats were kept in plastic cages with wire tops (120 × 70 × 60 cm), with one to two animals per cage. The cages were maintained in a well-ventilated room under a 12-hour light/dark cycle at a controlled temperature of 24–25 °C. During a seven-day acclimatization period, the rats had free access to drinking water and a standard Comfeed PARS diet *ad libitum*. Animals showing ≥10% body weight loss, abnormal behavior (e.g., signs of illness or anorexia), or mortality during the study were excluded. After acclimatization, the rats were randomly allocated to control and treatment groups and received the designated interventions according to the experimental protocol.

Sample and Research Groups

A total of 25 rats were randomly assigned to five groups. The normal control group (5 rats) received a single intraperitoneal injection of saline, in a volume equivalent to that administered to the alloxan-treated rats (corresponding to 150 mg/kg BW).[16] The untreated diabetic group (5 rats) were given a single intraperitoneal injection of alloxan at a concentration of 150 mg/kg BW. In the treated diabetic group I (5 rats), alloxan was administered at 150 mg/kg BW, and after 3 days the rats received once-daily administration of freeze-dried strawberries (FDS) at a dose of 1000 mg/kg BW via an orogastric tube for 4 week.[2] In the treated diabetic group II (5 rats), alloxan was given at the same dose, and after 3 days FDS was administered once daily at 1500 mg/kg BW via an orogastric tube for 4 weeks. Finally, the treated diabetic group III (5 rats) received alloxan at 150 mg/kg BW, and after 3 days were treated with once-daily FDS at 2000 mg/kg BW via an orogastric tube for 4 weeks.

Alloxan Injection

A 0.9% sodium chloride (NaCl) solution was used to dissolve alloxan at a concentration of 0.150 g/mL. A fresh preparation was made for every set of five rats. The solution was administered intraperitoneally to fasted animals using a single 1-mL tuberculin syringe, at a dose of 150 mg/kg body weight for each rat to induce diabetes.[16]

Freeze-dried strawberry (FDS)

Strawberries of the Mencir cultivar (*Fragaria* × *ananassa*) were harvested ripe from plantations managed by BUMDes Raharjo Lumbung Stroberi in Pandanrejo, a tourist village in Batu, Malang, East Java, Indonesia (S 07°52.117' E 112°32.526') on 15 June 2023. Taxonomic verification of the fruit samples took place at the Herbal Materia Medica Laboratory in Batu, Malang, East Java, with reference number 000.9.3/2633/102.20/2023 under the supervision of independent pharmacist Fitria Rahmawati, Head of the Herbal Laboratory Service. Following cleaning, the fruits were processed into cubic pieces measuring 50 × 50 × 50 mm³, sealed in opaque plastic containers, and held at –18 ± 2°C for seven days ahead of the freeze-drying step. The freeze-drying process utilized a LabFreez FD-12-MR apparatus (serial number FD2019090711), running at –50°C under 5 Pa pressure for 72 hours.[15] After being pulverized and weighed, the freeze-dried strawberry material was mixed into a 1% Carboxymethyl Cellulose Sodium (CMC-Na) vehicle. Each administered dose equivalent to 10% FDS in suspension, produced from 500 g fresh strawberries yielding 50 g FDS after freeze-drying. The FDS suspension (10% w/v in 1% CMC-Na) was administered once daily to the treatment groups via an orogastric tube, with the volume adjusted according to each rat's body weight and the experimental protocol.

Fasting Blood Glucose (FBG) Examination

The animals underwent an overnight food restriction of approximately 8–10 hours before glucose evaluation. Blood glucose was analyzed with a GlucoDR glucometer AGM-2100 device (Allmedicus, South Korea), following the operational guidelines provided by the manufacturer. Each rat was assessed twice: first, 72 hours after receiving alloxan to verify hyperglycemia, and again at the conclusion of the experimental period.[17] Rats were categorized as diabetic when their fasting glucose concentration was above 150 mg/dL. [18,19]

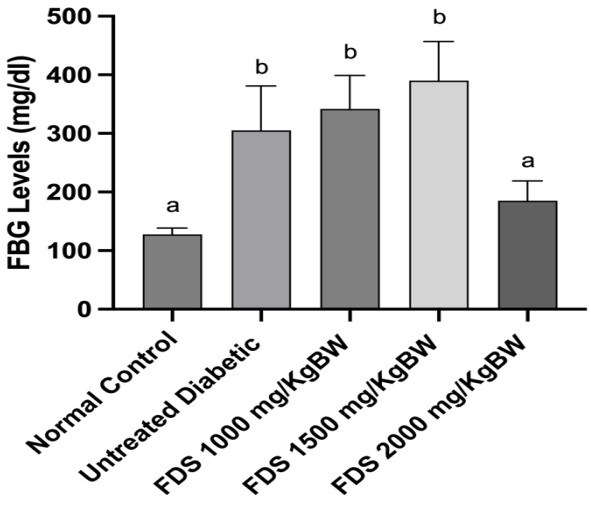

Figure 1: Fasting blood glucose levels were measured in normal control, untreated diabetic, and diabetic rats treated with FDS at doses of 1000, 1500, and 2000 mg/kg body weight (mg/kg BW). Data are presented as mean ± SD (n = 5 per group). Different letters indicate statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Games Howell post hoc test). FDS administration at 2000 mg/kg BW significantly reduced FBG compared to untreated diabetic rats. Data are presented as mean ± standard deviation (SD).

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Urinary Albumin-to-creatinine ratio (UACR) Examination

Urinary albumin and creatinine concentrations were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Rat ALB ELISA Kit, Elabscience®, Cat. No. E-EL-R3071; and Creatinine ELISA Kit, Elabscience®, Cat. No. E-EL-0058). Urine samples were collected in sterile tubes, centrifuged at 2,000 × g for 10 minutes at 4 °C to remove debris, and the supernatant was stored at −80 °C until analysis. Prior to assay, samples were thawed on ice and diluted appropriately (albumin 1:10; creatinine 1:5–1:10) to ensure that the concentrations fell within the standard curve range. All assays were conducted in duplicate following the manufacturer's protocols. For the albumin assay, 100 µL of standards, blanks, or samples were added to each well and incubated at 37 °C, followed by washing and sequential addition of biotinylated detection antibody, streptavidin–HRP, and TMB substrate. Absorbance was measured at 450 nm using a microplate reader, and albumin concentrations were interpolated from the standard curve. The creatinine assay was performed in a similar manner using the supplied enzyme conjugate and substrate reagents. The albumin-to-creatinine ratio (ACR) was calculated using the formula: ACR (mg/g) = urinary albumin (mg/L) / urinary creatinine (g/L). Results were expressed as mean ± standard deviation (SD) for each experimental group.

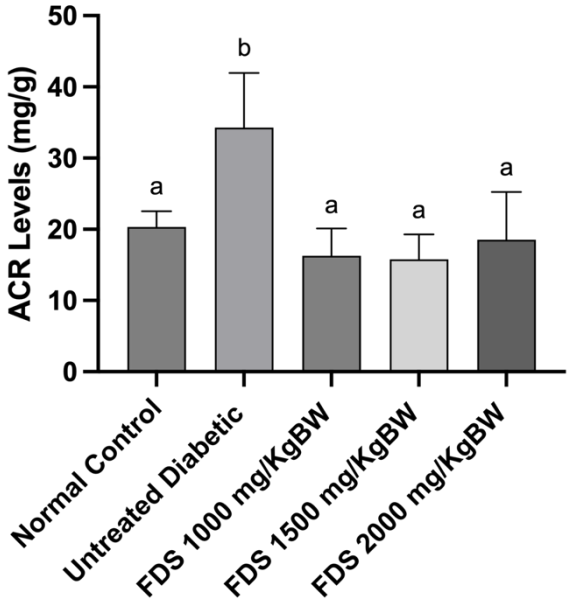

Figure 2: Albumin-to-creatinine ratio (ACR) levels were measured in normal control, untreated diabetic, and diabetic rats treated with FDS at doses of 1000, 1500, and 2000 mg/kg body weight (mg/kg BW). Data are presented as mean ± SD (n = 5 per group). Different letters indicate statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Games–Howell post hoc test). ACR levels were significantly increased in untreated diabetic rats compared to the normal control group, while FDS treatment at all tested doses markedly reduced ACR levels toward normal values. Data are presented as mean ± standard deviation (SD).

Renal Histopathology Examination

At the end of the 4-week treatment period, rats were deeply anaesthetised with ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), followed by euthanasia through decapitation.[17] The kidneys were immediately excised, rinsed with physiological saline, and fixed in 10% neutral-buffered formalin. Following fixation, the tissues were dehydrated through a graded ethanol series (70%, 80%, 95%, and 100%, each applied twice), cleared in xylene, and embedded in paraffin wax at approximately 60 °C. The paraffin blocks were allowed to harden and then sectioned at a thickness of 5 µm using a rotary microtome. Sections were mounted on glass slides and stained with haematoxylin and eosin (H&E) according to standard histological protocols.

Histopathological evaluation of the kidneys was performed under a light microscope at 400× magnification. Observations focused on renal tubules, including the proximal and distal convoluted tubules. For each animal, five randomly selected, non-overlapping fields of view were examined, located in the top left, bottom left, top right, bottom right, and central regions of the section. In each field, the numbers of damaged and total tubules were counted, and the percentage of tubular injury was calculated using the formula:[20]

$$\text{Tubular Injury (\%)} = \frac{\Sigma\text{Damaged Tubules}}{\Sigma\text{Total Tubules in FOV}} \times 100\%$$

The mean percentage across the five fields represented the overall degree of injury. Tubular damage was scored semi-quantitatively based on the proportion of affected tubules: 0 % (score 0, normal), 1–25 % (score 1, mild), 26–50 % (score 2, moderate), 51–75 % (score 3, severe), and > 75 % (score 4, extensive injury). Criteria for tubular injury included evidence of necrosis (pale or vacuolated cytoplasm, shrunken or absent nuclei, and pale or basophilic nuclear staining), epithelial cell desquamation, and thickening of the tubular basement membrane. The severity of necrosis was further graded according to cytoplasmic pallor and nuclear alterations, ranging from slightly pale cytoplasm with faintly basophilic nuclei to complete nuclear loss. The final histopathology score for each kidney was expressed as the mean value obtained from the five examined fields and used for subsequent statistical analysis.[20]

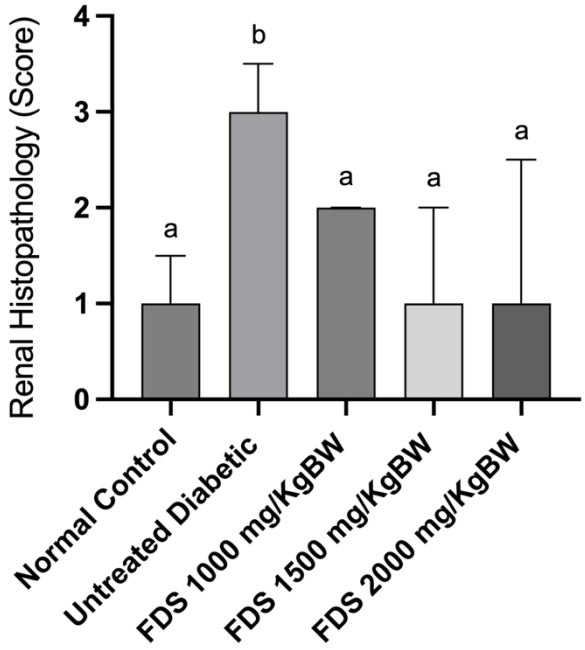

Figure 3: Renal histopathology scores were evaluated in normal control, untreated diabetic, and diabetic rats treated with FDS at doses of 1000, 1500, and 2000 mg/kg body weight (mg/kg BW). Data are presented as mean ± SD (n = 5 per group). Different letters indicate statistically significant differences among groups ($p < 0.05$, Kruskal–Wallis test followed by Mann–Whitney U post hoc analysis). Untreated diabetic rats exhibited significantly higher renal histopathology scores compared to the normal control group, while FDS treatment, particularly at higher doses, reduced renal tissue damage. Data are presented as Median (IQR).

Statistical Analysis

All continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or as median with a 95% confidence interval (CI) for non-normally distributed data. The normality of data was evaluated using the Shapiro–Wilk test. The distribution of fasting blood glucose and ACR measurements indicated normality, allowing

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group comparisons to be carried out using one-way ANOVA complemented by Tukey's post-hoc procedure. Non-normally distributed variables were analysed using the Kruskal–Wallis test followed by the Mann–Whitney U test. Statistical significance was set at $p < 0.05$. Data analysis was conducted using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA).

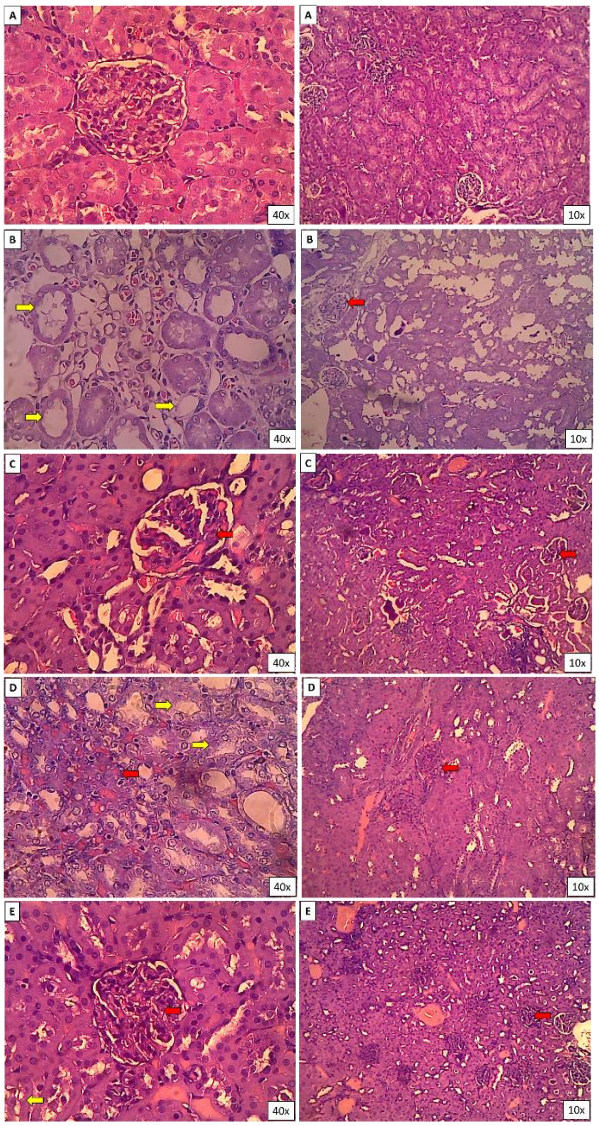

Figure 4: Renal Histopathology was assessed using kidney tubule damage scoring. **(A)** The normal control group had no kidney tubules damage (Score 0); **(B)** Untreated diabetic group has severely damaged kidney tubules (Score 5); **(C)** FDS 1000 mg/KgBW group had no kidney tubules damage (Score 0); **(D)** FDS 1500 mg/KgBW group shows moderate kidney tubules damage (Score 2); **(E)** FDS 2000 mg/KgBW group shows minimal kidney tubules damage (Score 1); kidney tubule damage (yellow arrow), glomerulus (red arrow).

Results and Discussion

Dose-Dependent Effects of Freeze-Dried Strawberries on Fasting Blood Glucose

The present study using a diabetic animal model demonstrated that alloxan injection effectively induced pancreatic dysfunction, leading to impaired insulin production and disrupted glucose metabolism. Alloxan and Streptozotocin (STZ) are two commonly used compounds for inducing diabetes in experimental animals. Alloxan, chemically known as 2,4,5,6-(1H,3H)-pyrimidinetetrone, is a well-established diabetogenic agent. The mechanism by which alloxan induces diabetes involves the selective targeting and destruction of pancreatic β-cells through a cascade of processes culminating in apoptosis.[21] Mechanistically, alloxan acts as a toxic glucose analogue, possessing a molecular structure similar to glucose that enables it to be taken up by β-cells via the GLUT2 transporter, where it exerts cytotoxic effects. Moreover, alloxan inhibits insulin secretion and promotes the generation of reactive oxygen species (ROS), leading to oxidative stress and β-cell damage. This sequence of events ultimately results in insulin deficiency, contributing to the development of insulin-dependent diabetes mellitus in experimental models.[17]

The results demonstrated a significant reduction in post-test fasting blood glucose (FBG) levels across all groups, as confirmed by one-way ANOVA analysis ($p < 0.01$). To ensure accurate assessment of FBG, mice were fasted for approximately 8–10 hours prior to sampling. These results confirm that the reduction in FBG levels is attributable to the administration of freeze-dried strawberries (FDS), as shown in Figure 1. Subsequent post hoc comparisons between the untreated diabetic group and the normal control group revealed a highly significant difference (305.4 ± 75.8 mg/dL vs. 127.6 ± 10.99 mg/dL; $p < 0.001$). However, treatment with 1000 mg/kg and 1500 mg/kg of FDS did not produce a significantly improvement compared with the untreated diabetic group (341.8 ± 57.05 mg/dL, $p = 0.823$; and 390.4 ± 66.57 mg/dL, $p = 0.135$, respectively). Interestingly, in the groups receiving 1000 mg/kg and 1500 mg/kg FDS (P1 and P2), FBG levels were elevated, likely due to the intrinsic glucose content of the FDS preparation, as shown in Figure 1. This effect may be attributed to the intrinsic sugar content of strawberries, which contributes to their sweetness and primarily consists of glucose, fructose, and sucrose.[22] In the presence of pancreatic dysfunction or insulin deficiency, these sugars can elevate blood glucose levels, counteracting the hypoglycaemic potential of strawberry-derived bioactive compounds.

Previous studies have reported that the glucose content in related strawberry cultivars ranges from approximately 19–43 g/kg for fructose and 13–26 g/kg for glucose.[22] Moreover, Zhan et al., (2019), demonstrated that oral glucose doses of 20 g/kg body weight induce glucose intolerance in mice, which may partly explain the marked increase in blood glucose observed in the 1000 and 1500 mg/kg FDS groups. These findings are consistent with previous studies,[2,24] which reported that low doses of strawberry extract failed to achieve optimal blood glucose reduction in diabetic animal models.

In contrast, the highest dose (2000 mg/kg) produced a marked reduction in FBG levels compared with the untreated diabetic group, approaching those observed in the normal control (185.2 ± 33.7 mg/dL vs. 305.4 ± 75.8 mg/dL; $p = 0.017$), as shown in Figure 1. The findings of this study also highlight the differences in outcomes among mice treated with FDS at doses of 1000, 1500, and 2000 mg/kg. The results demonstrated that administration of the 2000 mg/kg dose produced a significantly greater reduction in fasting blood glucose (FBG) levels compared with the lower doses.

Conversely, the highest dose used in this study (2000 mg/kg) produced a significant reduction in fasting blood glucose (FBG) levels. This finding indicates that at sufficient concentrations, the bioactive compounds present in strawberries, particularly pelargonidin, cyanidin, quercetin, and epicatechin, can enhance insulin production and promote glucose uptake.[15] The group receiving the 2000 mg/kg dose (P3) exhibited the lowest FBG levels compared with the other treatment groups. This effect may be attributed to the ability of *Fragaria* × *ananassa* (strawberry) to inhibit the enzymatic activities of lipase, α-amylase, and α-glucosidase, thereby reducing the conversion of polysaccharides and disaccharides into glucose and ultimately lowering circulating blood glucose concentrations. Strawberries lower fasting blood glucose (FBG) levels through several complementary mechanisms:[13] (1) the polyphenolic compounds enhance glucose metabolism and promote peripheral glucose uptake in insulin-sensitive tissues by increasing GLUT4 translocation and activity while reducing oxidative stress and inflammation; (2) anthocyanins improve insulin sensitivity by stimulating tyrosine phosphorylation of insulin receptors and upregulating the expression of insulin-regulated glucose transporters (GLUT4) in skeletal muscle, thereby enhancing insulin signalling and glucose transport; (3) anthocyanins also reduce insulin resistance through upregulation of GLUT4 gene expression, activation

of AMP-activated protein kinase (AMPK), and downregulation of retinol-binding protein 4 (RBP4), in addition to protecting pancreatic β-cells from streptozotocin-induced necrosis and inhibiting intestinal α-glucosidase and pancreatic α-amylase, thus reducing postprandial glucose absorption; and (4) strawberry extract helps restore the structural integrity of pancreatic β-cells, acts synergistically with insulin to alleviate hyperglycaemia, and enhances insulin secretion, altogether contributing to improved glycaemic regulation in diabetic conditions.

Freeze-Dried Strawberry Treatment Reduces ACR Levels Across All Doses

The study demonstrated a significant increase in albumin-to-creatinine ratio (ACR) levels among the groups ($p<0.01$), confirming the nephrotoxic effect of alloxan induction (normal ACR<30 mg/g). Post hoc analysis revealed a significant difference between the normal control and untreated diabetic groups (20.23 ± 2.21 vs. 34.26 ± 7.65 mg/g; $p = 0.03$). Furthermore, a consistent and significant reduction in ACR levels was observed in the groups treated with FDS at doses of 1000, 1500, and 2000 mg/kg compared with the untreated diabetic group (16.26 ± 3.8, $p<0.001$; 15.75 ± 3.5, $p < 0.001$; and 18.50 ± 6.7 mg/g, $p<0.001$ vs. 34.2 ± 7.6 mg/g, respectively), as shown in Figure 2. These findings indicate that FDS, even at relatively low doses, exerts nephroprotective effects in alloxan-induced diabetic rats by significantly lowering ACR levels.

Microalbuminuria is currently considered the gold standard for early detection of diabetic nephropathy. The diagnosis is commonly established through UACR testing on random spot urine samples. This method is recommended by major clinical practice guidelines such as the [25] and Kidney Disease Improving Global Outcomes (KDIGO), (2022). Monitoring UACR serves as a sensitive indicator of renal damage progression and therapeutic response in diabetes management. In the present study, changes in ACR were therefore evaluated to determine whether FDS supplementation could attenuate renal injury and improve kidney function in alloxan-induced diabetic rats.[25,26]

The results demonstrated a significant improvement in albumin-to-creatinine ratio (ACR) levels at all tested doses (1000, 1500, and 2000 mg/kg), as shown in Figure 2, indicating substantial restoration of renal glomerular structure and filtration function following FDS administration compared with the untreated diabetic group. These effects can be attributed to the antioxidant constituents of strawberries, including vitamins (such as vitamin C), β-carotene, and phenolic compounds such as phenolic acids, flavonoids, and anthocyanins.[27] The freeze-dried strawberry (FDS) used in this study was previously reported to contain abundant antioxidants, primarily kaempferol and quercetin, along with 88 other phytochemical compounds identified by liquid chromatography–mass spectrometry (LC-MS) analysis. Notably, freeze-dried strawberries exhibit higher antioxidant activity and greater total tannin, flavonoid, and polyphenol content compared to frozen strawberries; therefore, the rich antioxidant profile likely contributed to the observed nephroprotective effects, as evidenced by the improved ACR levels.[15] To date, only limited studies have reported similar findings, particularly regarding ACR modulation. Our study provides novel evidence that FDS exerts a nephroprotective effect by improving ACR in diabetic rat models.

This protective effect of FDS is likely mediated by the antioxidant constituents of strawberries, where the primary anthocyanin is pelargonidin-3-glucoside, followed by cyanidin-3-glucoside. In addition to anthocyanins, strawberries also contain potent antioxidants such as ellagic acid, quercetin, and kaempferol, which collectively contribute to the attenuation of oxidative stress, preservation of renal tubular integrity, and improvement in serum creatinine concentrations. These compounds act synergistically to scavenge reactive oxygen species (ROS), inhibit lipid peroxidation, and enhance endogenous antioxidant defences, thereby mitigating alloxan-induced renal injury. Antioxidants in strawberries, particularly polyphenols such as anthocyanins, flavonoids, ellagitannins, and vitamin C, help decrease the ACR in diabetic rat models through several mechanisms:[28,29] (1) by reducing oxidative stress, as strawberry bioactives lower reactive oxygen species (ROS) formation and lipid peroxidation markers such as malondialdehyde (MDA), while enhancing the activities of antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT), thereby protecting glomerular and tubular cells from oxidative injury and reducing albumin leakage into urine; (2) by improving insulin sensitivity through enhanced insulin signalling and increased expression of glucose transporter type 4 (GLUT4), leading to improved glucose uptake, reduced hyperglycaemia, and consequently diminished metabolic burden on the kidneys; (3) by exerting anti-inflammatory effects via downregulation of pro-inflammatory cytokines such as TNF-α and IL-6 and inhibition of the NF-κB signalling pathway, which together mitigate inflammation-induced renal damage and fibrosis; and (4) by preserving renal microstructure, as shown in histological findings where strawberry supplementation improves glomerular and tubular integrity in diabetic rats, thereby providing nephroprotection and delaying the progression of diabetic nephropathy. Antioxidant activity also has an antinephropathic effect by lowering blood glucose levels, anti-inflammatory biomarkers, lipid peroxidation, BUN, creatinine, uric acid, and urea in diabetic rats [30]. Additionally, animal studies using strawberry extract showed better reductions in serum creatinine and urea levels in diabetic models, supporting evidence for FDS as a nephroprotective agent.[2]

Freeze-dried Strawberries' Effects on Renal Histopathology

The histopathological evaluation of renal tissues revealed a significant difference among the groups. The untreated diabetic group that received alloxan induction exhibited a markedly higher renal damage score compared with the normal control (3.00 ± 0.70 vs. 0.80 ± 0.83; $p = 0.008$), as shown in Figure 3, indicating substantial tubular and glomerular injury. These findings reflect the direct cytotoxic effects of alloxan on renal cells and confirm the presence of microscopic renal damage in untreated diabetic rats, as shown in Figure 4B.

Furthermore, all FDS-treated groups at doses of 1000, 1500, and 2000 mg/kg showed a significant reduction in renal histopathology scores compared with the untreated diabetic group (1.40 ± 0.89, $p = 0.016$; 1.80 ± 1.30, $p = 0.013$; and 1.60 ± 0.89, $p = 0.037$ vs. 3.00 ± 0.71, respectively), as shown in Figure 3. These findings indicate that FDS at doses of 1000–2000 mg/kg body weight exerts nephroprotective effects in alloxan-induced diabetic rats by improving renal histological structure and reducing tissue damage, as shown in Figure 4.

The present study demonstrates the nephroprotective role of FDS in preventing the progression of microscopic renal injury, as evidenced by a significant reduction in kidney histopathology scores across all FDS-treated groups. These histological improvements, as shown in Figure 4, complement the functional findings observed in this study, particularly the significant improvement in albumin-to-creatinine ratio (ACR), further supporting the protective effects of FDS at the cellular and tissue levels.

These results are in agreement with previous experimental studies. Ibrahim & Maksoud, (2015) reported that strawberry leaf extract ameliorated histopathological alterations in the renal tubules and glomeruli of diabetic rats. Moreover, administration of strawberry fruit extract has been demonstrated to enhance kidney function in diabetic models.[2] These findings complement previous studies by Mallhi et al., (2023) and others, which did not clearly demonstrate histopathological alterations in renal tubules resulting from chronic hyperglycaemia following antioxidant treatment with astaxanthin. Moreover, the present results further confirm the nephroprotective potential of FDS at the cellular level, as reflected by improved renal architecture and functional restoration, supported by the significant improvement in ACR observed in this study.

The nephroprotective effects of FDS supplementation are likely attributed to its high content of polyphenols, flavonoids, and anthocyanins, each possessing potent antioxidant and anti-inflammatory activities. Polyphenolic compounds have been shown to attenuate inflammation, reduce mitochondrial oxidative stress, and improve renal microarchitecture in models of diabetic nephropathy.[29] Furthermore, freeze-dried berries preserve the bioactivity of phytonutrients, thereby contributing to improved metabolic parameters and target organ protection.[31]

Conclusion

Administration of freeze-dried strawberries (FDS) at a dose of 2000

mg/kg body weight significantly reduced fasting blood glucose (FBG) levels in alloxan-induced diabetic rats. Furthermore, FDS supplementation at doses of 1000, 1500, and 2000 mg/kg body weight effectively decreased the albumin-to-creatinine ratio (ACR) and improved renal histopathological scores compared with untreated diabetic controls. These findings demonstrate that FDS exerts pronounced antihyperglycaemic and nephroprotective effects, most likely attributable to its high content of antioxidant bioactive compounds, including flavonoids and polyphenols. Collectively, the results indicate that FDS supplementation may represent a promising natural adjunctive strategy for diabetes management by enhancing glycaemic control and preserving renal function. Nonetheless, further preclinical and clinical investigations are warranted to validate its therapeutic efficacy, clarify the underlying molecular mechanisms, and establish its long-term safety profile.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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