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Research Article

# Nephroprotective effects of *Equisetum ramosissimum* L. extract in streptozotocin-induced diabetic rats

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## Abstract

Diabetes is a widespread health issue that impacts people all over the globe. The *Equisetum ramosissimum* L. plant has numerous traditional uses and pharmacological properties, including antidiabetic effects.The objective of this study was to thoroughly examine the advantages of incorporating extracts from the aerial components of *E. ramosissimum* to control diabetic nephropathy. The phytochemical constituents of *E. ramosissimum* extract were explored using phytochemical and HPLC analysis, focusing on phenols and flavonoid content. The effect of plant extract was evaluated on different kidney function parameters linked to diabetic nephropathy (fasting blood glucose, creatinine, uric acid, and urea) in streptozotocin induced-diabetic rats. Histopathological changes in the liver were also examined. The results showed methanol and ethanol extracts of *E. ramosissimum* have a total content of phenols (equivalent to gallic acid, 7.62 and 8.97 mg/g) and flavonoids (equivalent to quercetin, 8.87 and 12.86 mg/g), respectively. After conducting the UHPLC-MS/MS analysis, it was found that both the methanol and ethanol extracts contained isoferulic acid, ISO-Orientin, myristic acid, linoelaidic acid, rutin, and 3-Glu-7-Rha quercetin. Additionally, isoferulic acid, myristic acid, linoelaidic acid, rutin, and 3-Glu-7-Rha quercetin were present in the extracts. The ethanol extract of *E. ramosissimum* significantly impacted STZ-induced diabetic mice by reducing their fasting blood glucose levels, and their creatinine and urea levels ($P < 0.005$). In conclusion, *E. ramosissimum* ethanol extract has shown potential effects to counteract some diabetes consequences on kidney function. Therefore, further studies are required to investigate its effect on other diabetes-related complications.

## Keywords

*Equisetum ramosissimum*, diabetes, nephroprotective, kidney functions, phenols, flavonoids

## Introduction

According to the WHO report (Hunt et al. 2021), diabetes mellitus affects a vast portion of the population globally. Therefore, there is an increased recognition of the importance of glycemic control and research in diabetes complications prevention (Barham et al. 2021).

The persistent complications of diabetes are broadly partitioned into microvascular and macrovascular complications, including diabetic nephropathy, which influences 20–30% of diabetic patients, with oxidative harm playing a critical part in its pathogenesis (Al-Waili et al. 2017).

Herbal and dietary supplements that are rich in flavonoids can potentially decrease the occurrence of diabetes. This effect is achieved by lowering blood sugar levels and offering other benefits that aid in the treatment and prevention of complications related to diabetes (Alkhalidy et al. 2018).

Some flavonoid compounds have shown preventive impacts on diabetic complications, such as hesperidin, naringenin, quercetin kaempferol, puerarin, and myricetin (Gandhi et al. 2020). The therapeutic effects of numerous components of flavonoids on diabetic nephropathy have been illustrated *in vitro* and *in vivo* studies, particularly apigenin, baicalein, and catechin, through anti-oxidation effects (Bai et al. 2019).

The study species *Equisetum ramosissimum* is called "horsetail" (Ang et al. 2015). Throughout history, this plant has been utilized as a diuretic, antitussive, astringent, and treatment for conditions such as ocular edema, diarrhea, jaundice, and renal lithiasis (Paulsamy et al. 2013). Other uses for treating urinary-related diseases, including stone formation in the kidney, stomachache, bone fracture, joint pains, and rheumatism, were also reported (Sureshkumar et al. 2021). The airborne parts of the *E. ramosissimum* were found to contain varied components of tannins, flavonoids, phenols, saponins, and alkaloids. It is widely recognized that these components are crucial in providing the plant extract with its antioxidant and antibacterial properties (Savaya et al. 2020). Interestingly, numerous quercetins and kaempferol derivatives were identified in the extract prepared from the ariel plant of *E. ramosissimum* (Wang et al. 2005). The latter are well known for their effect on glycemic control and preventing diabetes complications (Sivakumar et al. 2022).

Extracts from various Equisetum species were utilized in recent studies on diabetic rats. The results indicate that *E. arvense* exhibits high levels of phenolic and flavonoid compounds with potent antioxidant properties, making it a promising addition to diabetes therapy (Hegedűs et al. 2020). Researchers have established the correlation between kidney disorders and diabetes. The utilization of the species *E. myriochaetum* has been identified to combat these ailments (Angel et al. 2020). The efficacy of *E. giganteum*'s aqueous extract in controlling blood sugar levels has been scientifically proven (Vieira et al. 2020).

The main goal of this research was to explore the potential health benefits of different extract preparations from *E. ramosissimum* to prevent diabetes-related nephropathy. Multiple extracts of *E. ramosissimum* were created and analyzed to determine their antioxidant properties, concentration of phenols, and flavonoid content. The plant extract with optimal content of phytocomponents was investigated for its effect on different kidney function parameters linked to diabetes nephropathy, as it is considered a major complication of diabetes.

## Materials and methods

### Preparation of plant materials

For this study, the *E. ramosissimum* species was sourced from the Mujib reserve in Jordan. The plant species was authenticated by a knowledgeable botanist from the Royal Society for the Conservation of Nature (RSCN) with voucher number (*E. ramosissimum* 5/7/2017). The aerial components of the *E. ramosissimum* were cleaned, dried, and ground into smaller pieces using a blender.

### The extraction method

A range of extracts from the plant under study was obtained using the maceration method. The extracts were created by mixing 500 mL of either methanol or ethanol with 50 g of dry plant material at room temperature (25.0 °C) for 24 hours. Then, the resulting mixture was filtered with filter paper. Afterwards, the extracts were completely dehydrated using a rotary evaporator (R-300, Buchi, USA), at 60 °C and 90 rpm. Finally, each dried extract was accurately weighed to determine the extraction yield.

### Determination of total phenolic content

The total phenol content was determined through the Folin-Ciocalteu technique, as outlined in Sadeghi et al.'s publication (2015). A combination of 5 mL of plant extract solution (or gallic acid), Folin-Ciocalteu reagent, and 5% aqueous $Na_2CO_3$ was prepared. After a 30-minute incubation period, the phenolic concentration was measured calorimetrically using Spectrophotometer (Hitachi U-1800, UK) at 765 nm. The total phenolics (mg/mL) amount was subsequently calculated as the gallic acid equivalent.

### Identification of phenols and flavonoids using UHPLC/MS-MS

After dissolving each extracted sample in 2 mL of DMSO and up to 50 ml of acetonitrile, they were centrifuged at 4000 rpm for 2.0 min. Then, 3.0 µL of each sample containing 1.0 ml was injected into the autosampler.

A Bruker Daltonik (Bremen, Germany) Impact II electrospray ionization quadrupole time of flight (ESI-Q-TOF) system equipped with a Bruker Daltonik Elute Ultra-Performance Liquid Chromatography (UPLC) system

(Bremen, Germany) were thoroughly utilized to test specific compounds against an integrated library. Chromatography was employed to separate the compounds, and they were analyzed for m/z and the retention time using highly precise Bruker TOF MS and standards.

Chromatographic separation was performed with the utmost precision using the Bruker solo 2.0 C-18 UHPLC column (100 mm × 2.1 mm × 2.0 m) at a consistently reliable flow rate of 0.51 mL/min and a stable column temperature of 40 °C. The solvents used, a mixture of water containing 0.05% formic acid (A) and acetonitrile (B), were carefully selected to optimize the results. B's method was skillfully employed for a 35-minute analysis with gradient elution (80–95%), ensuring the accuracy and efficiency of the process.

## The experimental study design

### *Animals*

Wistar rats weighing 150–200 grams and aged 70 days received a regular diet and had unrestricted water access from Hammoudeh business in Amman, Jordan. The experiments on rats were conducted in accordance with the Declaration of Helsinki. Before the experiment, the rats were acclimated to the animal housing conditions for ten days, including a temperature range of 21–23 °C and a 12-hour light-dark cycle. The Institutional Committee on Ethics of Animal Experimentation approved the procedure. All procedures were performed in accordance with international regulations for the care and use of laboratory animals. Ethical approval for the study was obtained by the Institutional Review Board at Applied Science Private University, Amman, Jordan, Approval Number: 2021-PHA-40.

### *Induction of diabetes in rats*

The rats were given two doses of freshly dissolved streptozotocin (STZ, Sigma, USA). through intraperitoneal (IP) administration. A total of 55 mg/kg of STZ was given in citrate buffer (0.01 M, pH 4). The first dose was 30 mg/kg, followed by a second dose of 25 mg/kg on the third day after 48 hours. After each STZ dose, the rats were returned to their cages and provided unrestricted food and drink access (5% dextrose to prevent severe hypoglycemia). Control rats were given citrate buffer.

After 72 hours of administering STZ, the rats were confirmed to have diabetes (STZ-DM: STZ-induced diabetes). A commercial glucose kit measured their blood glucose levels Qualigens Diagnostics – AccuChek- (ROCHE, TD-4277, Augsburg, Germany). The test of the blood sample from the rat's tail was applied twice on each test-strip lot. If the fasting blood glucose (FBG) level was 250 mg/dL or higher (Al-Matubsi et al. 2016), we classified the rat as diabetic, following Nagasawa et al.'s (2003) criteria. The body weight of each rat was measured weekly as part of their follow-up.

Two more samples were collected and analyzed alongside the blood droplets to confirm and monitor STZ-DM as follows:

1. Three days after the STZ injection and before the therapy, the blood samples were collected from the rats. The retro-orbital plexus was accessed to collect 0.2 mL from each animal into heparinized tubes. The collected plasma was centrifuged for 10 minutes at 2000 rpm and tested for FBG.
2. The animals had blood samples taken at the end of the 21-day experiment. Each sample had a 2–3 ml volume and was stored in tubes with heparin. The tubes were allowed to coagulate for an hour and then centrifuged at 4000 rpm for 10 minutes. At least 1 ml of blood was transferred into Eppendorf tubes to extract serum, labelled accordingly. All samples remained stored at -80 °C until they were ready for biochemical testing.

## Plant extract solution preparation and administration

The dried plant extract was dissolved in distilled water at 100 or 150 mg/ml concentrations to create fresh ethanol *E. ramosissimum* extract stock solutions. Each was given orally to rats through an intragastric tube, with a dose of 200 or 300 mg/kg BW, like a prior research study (Carneiro 2013). The *E. ramosissimum* extract treatments began on the third day after STZ injection, and the experiment was conducted for three weeks on the same days after diabetes was induced.

### *Animal groups*

A detailed investigation examined the potential benefits of *E. ramosissimum* extract in preventing diabetes and protecting against kidney damage. The study involved forty-two rats, divided into seven groups of six animals, each as follows:

**Group (C):** non-diabetic control group.
**Group (SDM):** STZ-induced diabetic rats (55 mg/kg).
**Group (SDMET):** STZ-induced diabetic rats, orally treated with the standard antidiabetic drug metformin (50 mg/kg/day).
**Group (Q2):** Non-diabetic rats received *E. ramosissimum* ethanol extract (200 mg/kg/day).
**Group (SDMQ2):** STZ-induced diabetic rats received *E. ramosissimum* ethanol extract (200 mg/kg/day).
**Group (Q3):** Non-diabetic rats received *E. ramosissimum* ethanol extract (300 mg/kg/day).
**Group (SDMQ3):** STZ-induced diabetic rats received *E. ramosissimum* ethanol extract (300 mg/kg/day).

### *Biochemical analysis*

The rats were not fed the night before they were euthanized on the 21st day of the experiment. Blood samples were collected before and after the experiment to produce serum, which was then analyzed using liquicolor-HUMAN kits to measure the levels of FBG, creatinine (Cr), uric acid (UA), and urea (U).

### *Histopathological studies*

The kidney slices, fixed in formalin and embedded in paraffin wax, were stained using Hematoxylin and Eosin (H

& E) at a thickness of 4 micrometers. The sections were thoroughly examined under a Leica microscope (The Life Sciences companies at Danaher Corporation ("Danaher"), Germany) and captured using a Leica MC 170 HD camera utilizing LAS EZ software.

### *Statistical analysis*

Version 27.0 of the Statistical Package for the Social Sciences (SPSS) for Windows, developed in Chicago, IL, USA, was utilized to perform the statistical analysis.

The one-way ANOVA test was used to identify any significant differences in the average values of each parameter among different experimental groups. The post hoc multiple comparisons with Dunnett's Multiple Comparisons tests were used to analyze any meaningful variations in mean values between the experimental and control groups.

The study aimed to thoroughly examine the potential impact of FBG as a predictor of diabetes mellitus and Cr, U, and UA as predictors of diabetes nephropathy from the beginning to the end of the trial. Using a stepwise approach to multiple linear regression, researchers determined the factors that played a crucial role in the relationships between diabetes and diabetic nephropathy across various research groups.

## Results

### Plant sample extraction

The extraction yield was computed using % w/w dry weight. Based on the data, it was found that ethanol produced the highest output at 3.42%, followed closely by methanol at 2.78%.

### Determination of total phenolic content

Based on the analysis of total phenol content, it has been determined that the methanol and ethanol extracts contain 7.62 and 8.97 mg/g, equivalent to gallic acid, respectively.

### Determination of total flavonoid content

Upon evaluating their total flavonoid content, it has been determined that the methanol and ethanol extracts possess 8.87 and 12.86 mg/g of quercetin, respectively.

### Identification of phenols and flavonoids using UHPLC/MS-MS

Identification of the phytocomponents present in the methanol and ethanol extracts of *E. ramosissimum* was made possible using UHPLC/MS-MS. Tables 1, 2 display the identified substances, and Figs 1, 2 showcase the chromatograms for the recognized chemicals in the methanol and ethanol extracts, respectively. The methanol extract contained six substances, namely isoferulic acid, ISO-Orientin, myristic acid, linoelaidic acid, rutin, and 3-Glu-7-Rha quercetin. The ethanol extract, on the other hand, had these six substances and eight additional ones, which include 7-Methoxy-4'-hydroxyflavone, 7-Glu Chrysoeriol, and succinic acid.

**Table 1.** Phytochemicals detected in *E. ramosissimum* methanol extract using UHPLC/MS-MS.

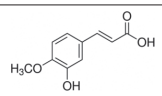
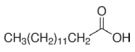
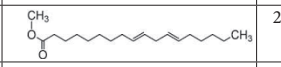
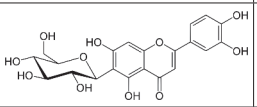
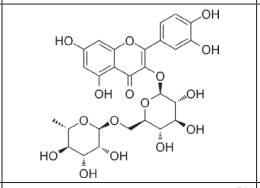
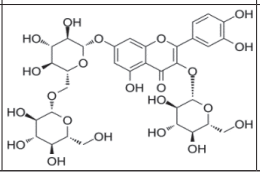
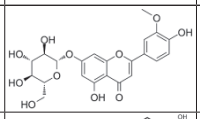
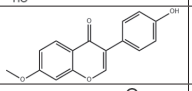
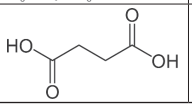
| Compound name | Structure | RT [min] | m/z |
|---|---|---|---|
| 3-Hydroxy-4-methoxycinnamic acid (Iso ferulic acid) |  | 5.39 | 173.16261 |
| Myristic acid |  | 27.14 | 181.18303 |
| Linoelaidic acid |  | 29.82 | 213.26471 |
| ISO-Orientin |  | 4.88 | 257.37702 |
| Rutin |  | 5.6 | 277.42807 |
| 3-Glu-7-Rha Quercetin |  | 5.25 | 281.43828 |

**Table 2.** Phytochemicals detected in *E. ramosissimum* ethanol extract using UHPLC/ MS-MS.

| Compound name | Structure | RT [min] | m/z |
|---|---|---|---|
| 7-Glu Chrysoeriol |  | 7.1 | 261.38723 |
| 7-Methoxy-4'-hydroxyflavone |  | 11.43 | 193.21366 |
| Succinic acid |  | 0.98 | 117.01967 |

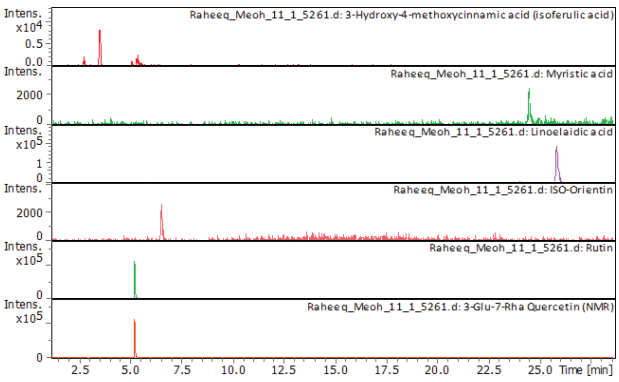



**Figure 1.** Chromatogram of *E. ramosissimum* methanol extract.

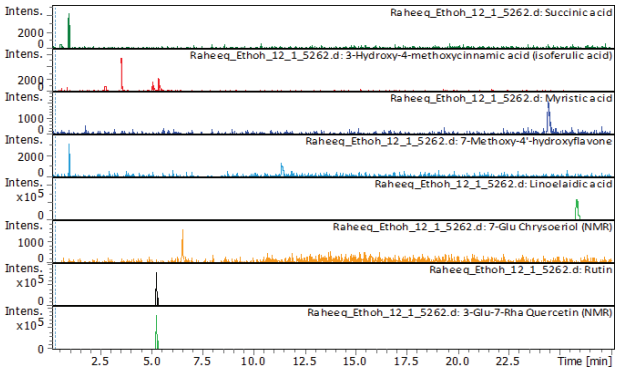



**Figure 2.** Chromatogram of *E. ramosissimum* ethanol extract.

## Biochemical changes within study groups at baseline and the end of the study period

### *Changes in body weight*

During the study, all groups, except the control group, showed a considerable reduction in their average body weight at the beginning and end of the research. This fact is undeniably supported by the *P*-value of 0.05, as shown in Table 3. Notably, the non-diabetic groups, which received the extract (Q2 and Q3), experienced a lower percentage of weight loss, which is a significant observation to consider.

**Table 3.** Changes of BW at baseline and the end of experiment in all study groups.

| Body weight (g) | | | | |
|---|---|---|---|---|
| **Group (n=6)** | **Baseline** | **End of the study** | **Change (%)** | ***P*[a]** |
| C | 178.3±12.2 | 190.8±10.7 | 12.50 (7.01%) | < 0.001 |
| SDM | 210±22.6 | 142.5±24.7 | -67.50 (-32.14%) | < 0.001 |
| SDMET | 200±16.1 | 166.7±8.2 | -33.33 (-16.67%) | 0.06 |
| Q2 | 205±5.5 | 192.5±5.2 | -12.5 (-6.01%) | < 0.001 |
| SDMQ2 | 221.7±15.1 | 152.5±32.1 | -69.17 (-31.20%) | 0.002 |
| Q3 | 187.5±10.4 | 175±10.5 | -12.5 (-6.67%) | < 0.001 |
| SDMQ3 | 213.3±15.4 | 167.5±26.2 | -45.83 (-21.5%) | 0.001 |
| F | 6.367 | 5.419 | | |
| *P*[b] | < 0.001 | | | |
| *P*[c] | | < 0.001 | | |

**Notes:** *P*[a] = *P*-value of difference between baseline and end of the study, *P*[b] = *P*-value of baseline, *P*[c] = *P*-value at the end of the study, F = F-test.
**Abbreviations:** C = non-diabetic control group, SDM = STZ-induced diabetic rats, SDMET = STZ-induced diabetic rat + metformin (500 mg/kg/day), Q2 = Low dose extract (200 mg/kg/day), SDMQ2= STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats + High dose extract (300 mg/kg/day).

### *Changes in fasting blood glucose*

Based on the results shown in Table 4, it appears that both doses of the extract given to the diabetic groups (SDMQ2 and SDMQ3) had a significant impact on FBG levels, comparable to the effect of metformin (SDMET) by the end of the study period (*P*-value < 0.05). However, the extract-treated non-diabetic groups (Q2 and Q3) only displayed a minor non-significant effect on FBG, although there was a significant effect on the retention of BW for these groups. This finding suggests that the extract may have a more substantial effect on diabetic models than normal animals.

**Table 4.** Changes of FBG at baseline and the end of the experiment in all study groups.

| Fasting Blood Glucose (FBG) (mg/dl) | | | | |
|---|---|---|---|---|
| **Group (n=6)** | **Baseline** | **End of the study** | **Change (%)** | ***P*[a]** |
| C | 93.90±5.04 | 93.78±3.84 | -0.06 (-0.06%) | 0.955 |
| SDM | 487.8±40.4 | 501.7±64.6 | 43.83 (8.99%) | 0.104 |
| SDMET | 326±85.8 | 81.2±27.4 | -244.78 (-75.08%) | < **0.001** |
| Q2 | 91±8.2 | 90.9±9.8 | -0.13 (-0.14%) | 0.979 |
| SDMQ2 | 423±115.7 | 132.7±55.6 | -290.3 (-68.7) | |
| Q3 | 93.4±14.5 | 101.1±8.2 | 7.72 (8.27%) | 0.369 |
| SDMQ3 | 259.2±74.7 | 155.93±78.1 | -103.27 (-39.8) | **0.002** |
| F | 37.93 | 66.714 | | |
| *P*[b] | < 0.001 | | | |
| *P*[c] | | < 0.001 | | |

**Notes**: *P*[a] = *P*-value of difference between baseline and end of the study, *P*[b] = P-value of baseline, *P*[c] = *P*-value at the end of the study, F = F-test.
**Abbreviations:** C = non-diabetic control group, SDM = STZ-induced diabetic rats, SDMET = STZ-induced diabetic rats + metformin (50 mg/kg/day), Q2 = Low dose extract (200 mg/kg/day), SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats + High dose extract (300 mg/kg/day).

### *Changes in urea levels*

As shown in Table 5, the mean serum levels of urea were significantly decreased in *E. ramosissimum* extract-treated groups. This finding was the opposite of the metformin effect observed at the end of the experiments, as supported by the paired T-test analysis.

Fig. 3 shows that the mean U levels for diabetic animals treated with Q2 and Q3 were considerably lower than those treated with metformin (SDMET, $P < 0.001$). This conclusion is drawn from post-hoc multiple comparisons utilizing Dunnett's Multiple Comparisons Test.

**Table 5.** Changes in urea (U) at baseline and the end of the experiment in all study groups.

| Urea (U) (ng/ml) | | | | |
|---|---|---|---|---|
| **Group (n=6)** | **Baseline** | **Follow-up** | **Change (%)** | ***P*[a]** |
| C | 37.7±4.04 | 37.5±5.14 | -0.2 (-0.53%) | 0.91 |
| SDM | 42.6±5.2 | 43.1±0.2 | 0.5 (1.17%) | 0.71 |
| SDMET | 43±9.3 | 53.1±9.5 | 10.02 (23.3%) | **0.012** |
| Q2 | 40.3±2.6 | 34±4 | -6.23 (-15.46%) | **0.007** |
| SDMQ2 | 43±9.34 | 32.5±4.9 | -10.6 (-24.65%) | **0.013** |
| Q3 | 39.8±1.9 | 29.9±3.4 | -9.93 (-24.25%) | **0.003** |
| SDMQ3 | 30.6±5.2 | 30.6±11 | 0.00 (0%) | 0.995 |
| F | 4.6 | 9.2 | | |
| *P*[b] | 0.002 | | | |
| *P*[c] | | < 0.001 | | |

**Notes:** *P*[a] = *P*-value of difference between baseline and follow-up, *P*[b] = P-value of baseline, *P*[c] = *P*-value of fallow up, F = F-test.
**Abbreviations:** C = non-diabetic control group, SDM = STZ-induced diabetic rats, SDMET = STZ- induced diabetic rat + metformin (50 mg/kg/day), Q2= Low dose extract (200 mg/kg/day), SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats + High dose extract (300 mg/kg/day).

### *Changes in creatinine levels*

As presented in Table 6, the study found a significant difference in mean Cr serum level between the control and experimental groups ($F = 7.301$, $P^c < 0.001$). Post-hoc multiple comparisons by Dunnett, showed that both healthy and diabetic-treated (Q3) animals exhibited lower mean Cr levels ($p < 0.01$) than in the diabetic control animal group (SDM) as shown in Fig. 4.

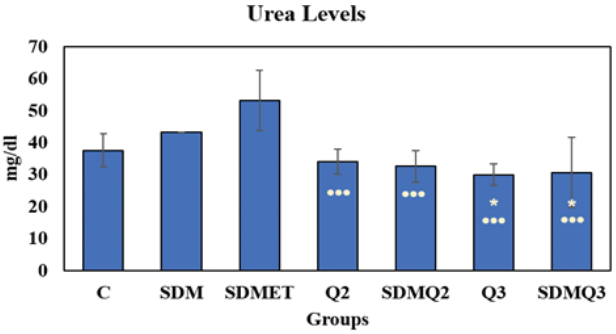



**Figure 3.** Final Mean values of Urea serum levels at the end of the study. **Abbreviations:** C = non-diabetic control group, SDM = STZ-induced diabetic rats, SDMET = STZ-induced diabetic rat + metformin (50 mg/kg/day), Q2 = Low dose extract (200 mg/kg/day), SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats + High dose extract (300 mg/kg/day). **NOTE**: Results are represented as Mean±SD ($n$=6 for each group). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ when compared with the SDM; • $p < 0.05$, •• $p < 0.01$, and •••$p < 0.001$ when compared with the SDMT (Dunnett's Multiple Comparisons Test).

### *Changes in UA levels*

Table 7 reveals that following the investigation, the SDMQ3 group experienced a noteworthy reduction in their mean blood UA levels ($P< 0.01$), with no other significant alterations detected.

## Stepwise regression analysis

**Table 6.** Changes in mean creatinine (Cr) levels at baseline and the end of the experiment in all study groups.

| Creatinine (Cr) (ng/ml) | | | | |
|---|---|---|---|---|
| **Group (n=6)** | **Baseline** | **Follow-up** | **Change (%)** | ***P*[a]** |
| C | 0.44±0.05 | 0.43±0.08 | -0.08 (-18.18%) | 0.94 |
| SDM | 0.51±0.07 | 0.62±0.07 | 0.11 (21.57%) | 0.05 |
| SDMET | 0.44±0.05 | 0.55±0.05 | 0.11 (25%) | **0.035** |
| Q2 | 0.43±0.04 | 0.50±0.02 | 0.08 (18.60%) | **0.004** |
| SDMQ2 | 0.62±0.07 | 0.48±0.06 | -0.13 (-20.97%) | **0.001** |
| Q3 | 0.44±0.02 | 0.43±0.04 | -0.02 (-4.54%) | 0.413 |
| SDMQ3 | 0.51±0.07 | 0.42±.0.12 | -0.09 (-17.65%) | 0.278 |
| F | 8.589 | 7.301 | | |
| *P*[b] | < 0.001 | | | |
| *P*[c] | | < 0.001 | | |

**Notes:** *P*[a] =*P*-value of difference between baseline and follow-up, *P*[b] = *P*-value of baseline, *P*[c] = *P*-value of fallow up, F = F-test.
**Abbreviations:** C= non-diabetic control group, SDM= STZ induced diabetic rats, SDMET = STZ-induced diabetic rat + metformin (50 mg/kg/day), Q2 = Low dose extract (200 mg/kg/day), SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats + High dose extract (300 mg/kg/day).

The findings from Table 8 conclusively showed that certain variables significantly impacted body weight, U, and FBG serum levels, as revealed by the regression analysis.

The stepwise regression analysis showed significant associations between IDVs such as FBG, BW, and Cr levels.

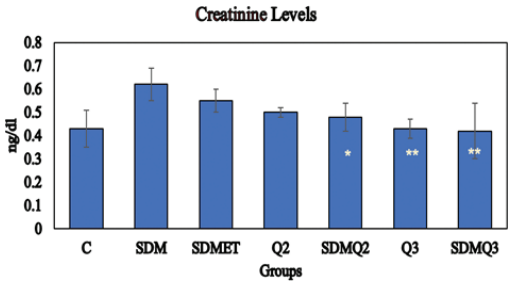



**Figure 4.** Final Mean values of Urea serum levels at the end of the study. **Abbreviations:** C = non-diabetic control group, SDM = STZ induced diabetic rats, SDMET = STZ-induced diabetic rat + metformin (50 mg/kg/day), Q2 = Low dose extract (200 mg/kg/day), SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats+ High dose extract (300 mg/kg/day). **NOTE**: Results are represented as Mean±SD ($n$=6 for each group). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ when compared with the SDM; • $p < 0.05$, •• $p < 0.01$, and •••$p < 0.001$ when compared with the SDMT (Dunnett's Multiple Comparisons Test).

**Table 7.** Changes in Uric acid (UA) at baseline and the end of the experiment in all study groups.

| Uric Acid (UA) (ng/ml) | | | | |
|---|---|---|---|---|
| **Group (n=6)** | **Baseline** | **Follow-up** | **Change (%)** | ***P*[a]** |
| C | 1.17±0.13 | 1.34±0.18 | 0.17 (14.52%) | 0.64 |
| SDM | 1.67±0.11 | 1.86±0.37 | 0.19 (11.38%) | 0.352 |
| SDMET | 1.17±0.13 | 1.18±0.39 | 0.01 (0.85%) | 0.948 |
| Q2 | 1.11±0.09 | 1.51± 0.51 | 0.41 (36.94%) | 0.126 |
| SDMQ2 | 1.86±0.37 | 1.72±0.26 | -0.14 (-7.53%) | 0.234 |
| Q3 | 1.20±0.16 | 1.21±0.25 | 0.01 (0.003%) | 0.948 |
| SDMQ3 | 1.67±0.11 | 1.00±0.24 | -0.67 (-40.12%) | **0.003** |
| F | 17.980 | 5.858 | | |
| *P*[b] | < 0.001 | | | |
| *P*[c] | | < 0.001 | | |

**Notes:** *P*[a] = *P*-value of difference between baseline and follow-up, *P*[b] = P-value of baseline, *P*[c] = *P*-value of fallow up, F = F-test.
**Abbreviations:** C = non-diabetic control group, SDM = STZ-induced diabetic rats, SDMET = STZ-induced diabetic rat + metformin (50 mg/kg/day), Q2 = Low dose extract (200 mg/kg/day), SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), Q3 =: High dose extract (300 mg/kg/day), SDMQ3 = STZ-induced diabetic rats + High dose extract (300 mg/kg/day).

In addition, the influence of BW on UA levels was also observed. During follow-up, the multiple linear regression model explained a considerable 95% and 66.4% of the variation in Cr and UA, respectively. The SDMQ3 showed

**Table 8.** The multivariate associations between the experiment variables using stepwise regression at the end of the experiment (3 weeks).

| Dependent variable | Study group | Univariate effect estimate | Coefficient | | | | |
|---|---|---|---|---|---|---|---|
| | | | **B** | **F** | **R** | **$R^2$** | ***P*-value** |
| Cr | SDMQ2 | FBG | 0.002 | 13.134 | 0.876 | 0.767 | 0.022 |
| | | BW | 0.001 | 26.723 | 0.973 | 0.947 | 0.012 |
| UA | SDMQ2 | BW | -0.007 | 7.901 | 0.815[b] | 0.664 | 0.048 |
| | SDMQ3 | U | 0.02 | 21.964 | 0.920 | 0.846 | 0.009 |

**Note**: B = slope; F = variation between sample means/variation within the samples; R = Pearson linear correlation coefficient; $R^2$ = determinant coefficient.
**Abbreviations:** SDMQ2 = STZ-induced diabetic rats + Low dose extract (200 mg/kg/day), SDMQ3 = STZ-induced diabetic rats (300 mg/kg/day) + High dose extract (300 mg/kg/day), U = urea, UA = uric acid, FBG = fasting blood glucose, Cr = creatinine, BW = body weight.

a robust reciprocal association between U and UA levels, with a whopping 85% of the variation in UA or U levels at follow-up fully explained by the model.

## Histopathological studies

The control group's rat liver tissue samples underwent light microscopy analysis to reveal the typical histological architecture of the hepatocytes, central vein, sinusoids, hepatic triads, and Kupffer cells. However, diabetic rats' liver tissue samples exhibited necrotic hepatocytes with lymphocyte infiltration, disorganized sinusoids, and dilated central veins. It is important to note that compared to metformin-treated rats, diabetic rats treated with the extract showed significantly reduced necrosis in the liver, as seen in Fig. 5. At the same time, the metformin-treated rats showed abnormally shaped hepatocytes and dilated veins, as seen in Fig. 5.

Under light microscopy, the kidney sections of control rats displayed regular Bowman's capsule, proximal tubules, and distal tubules. However, STZ-treated diabetic rats showed renal abnormalities, including glomerular tuft atrophy, renal tubule degeneration, and an increase in Bowman's gap size, as well as hemorrhage and renal blood vessel congestion. Despite most administered Bowman's capsules to diabetic rats with typical kidney damage, some distal tubules exhibited widening. Regrettably, metformin did not provide any protection against STZ-induced kidney damage, as shown in Fig. 6.

# Discussion

The Jordanian herbal treatments for diabetes were thoroughly examined by Issa et al. (2019), and the following plants were found to be effective: *Artemisia vulgaris*, *Lepidium sativum*, *Astragalus onbrgchis*, *Olea europaea*, *Rheum ribes*, *Teucrium polium*, *Salvia triloba*, and *Cinnamomum ceylanicum*. It is important to note that *Fucus vesicolosus* has significantly affected diabetes. Previous studies examined medicinal plants like *Zea mays* L. (Poaceae) for diabetes treatment in Jordan. The study found that Zea mays L. can lower blood glucose levels and protect against diabetic nephropathy in rats with STZ-induced diabetes (Afifi and Kasabri 2013).

According to preclinical studies, it is evident that *E. ramosissimum* ethanol extract (Al-Bayati et al. 2023) and *E. myriochaetum* aqueous extract (Cetto et al. 2000) possess hypoglycemic effects that are comparable to the well-known antidiabetic medications metformin and glibenclamide, respectively, in STZ-diabetic rats. In this study, findings noted that *E. ramosissimum* ethanolic extract is considered a potential agent used to prevent diabetic nephropathy associated with diabetes.

This study has shown that *E. ramosissimum* extract has similar effects to metformin in reducing FBG, U and Cr levels in diabetic and normal rats. It is noteworthy that previous studies have identified several *Equisetum* plant species that protect against adverse impacts on renal function in diabetic rats (Boeing et al. 2021).

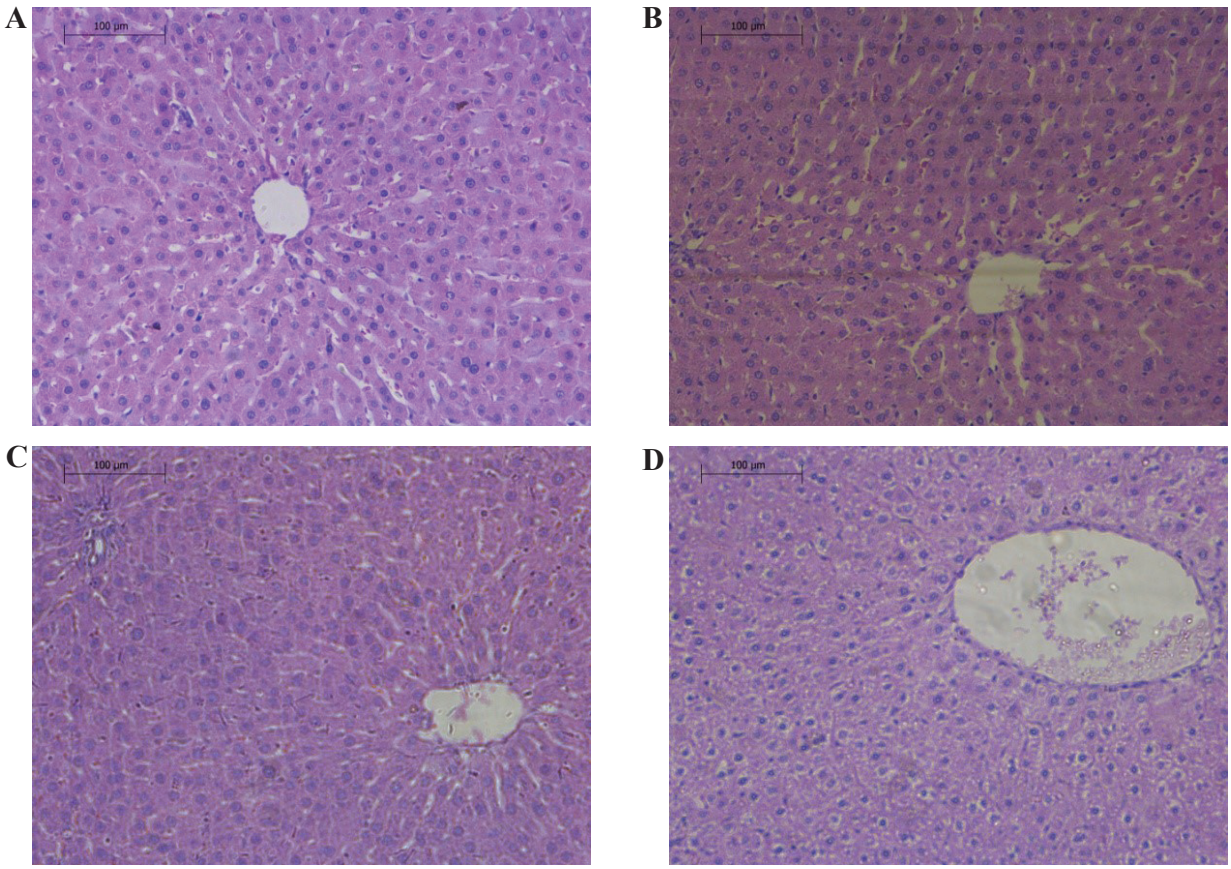



**Figure 5.** Liver tissue sections of (**A**) control; **B.** STZ-treated diabetic-rat; **C.** Extract-treated rats; **D.** Metformin-treated rats. White arrows: Central vein. Black arrows: Sinosoids. H& E stain.

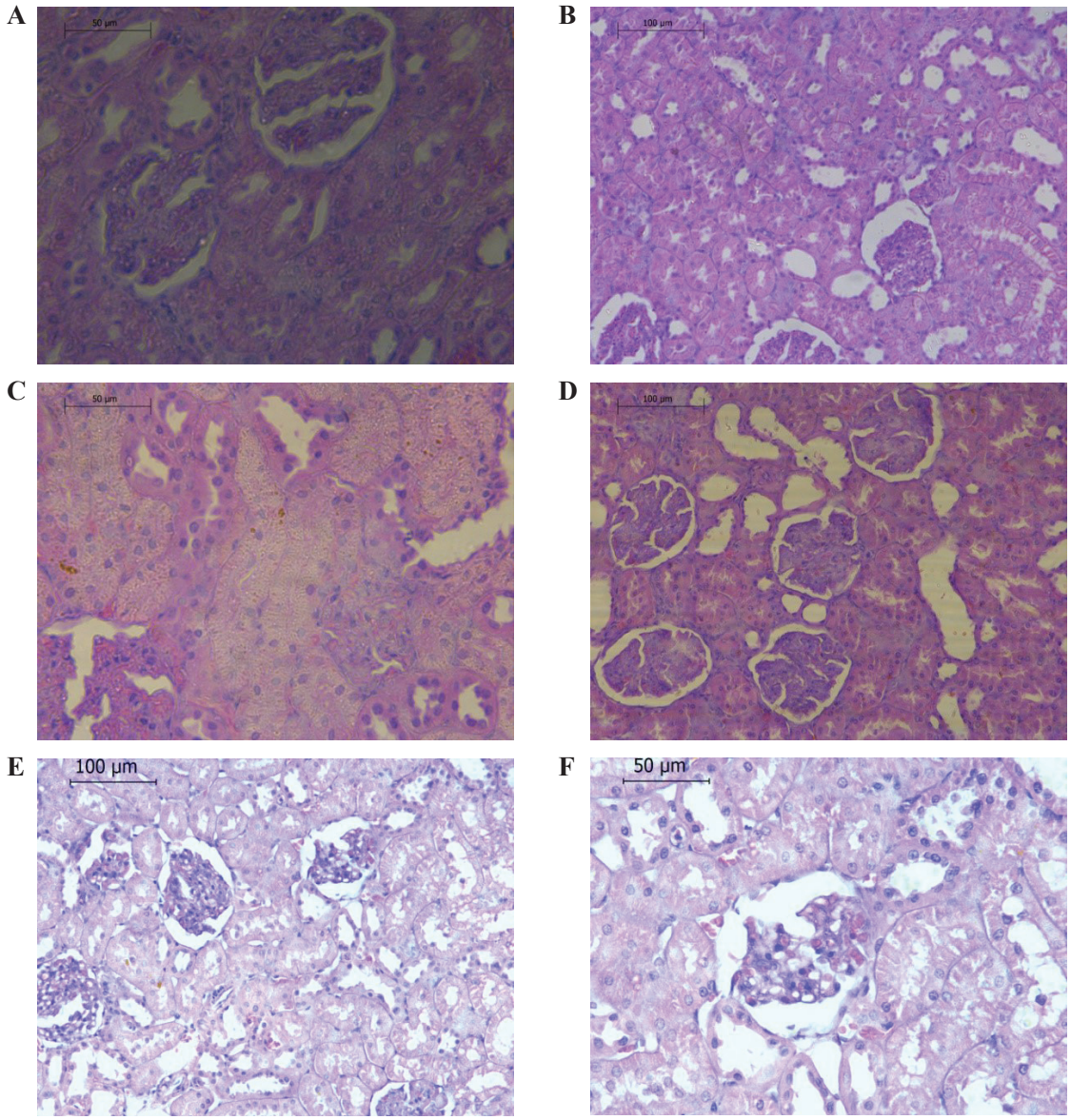



**Figure 6.** Kidney sections (**A**) control. **B, C.** STZ-treated diabetic-rat showing atrophy of glomerular tuft (white arrow in **B** arrow), increase in the Bowman's space area and degeneration of renal tubules (Black arrow in **C**) associated with severe hemorrhage and congestion of renal blood vessels; **D.** Extract-treated rats. Renal tubules (Black arrow), Bowman's capsule (white arrow); **E, F.** Metformin-treated rats. H & E stain.

In SDMQ2 group, significant reduction ($P< 0.05$) in the means serum U and Cr levels were observed, while means serum UA levels was reduced in SDMQ3 only. These findings may point to potential powerful antioxidant activity for the extracts, but higher doses as in SDMQ3 study group might have an adverse or toxic effects (Banerjee et al. 2003) .

Furthermore, the same group (SDMQ2) practically showed similar effect to that observed in diabetic -metformin treated group (SDM). Accordingly, in the term of the potential anti-DN effects for *E. ramosissimum* extract observed in SDMQ2 group , its bioactive compounds are likely to have therapeutic outcomes . Based on the stepwise regression findings in these experiments, the bioactive compound in the extract is expected to act peripherally rather than centrally. in this context, it has shown that the intervention of *E. myriochaetum* extract significantly reduced FBG levels without significant changes in the insulin levels in type 2 diabetic patients (Revilla et al. 2002). Significant reduction in BW observed in animal groups treated with *E. ramosissimum* extracts might also be additional evidence on the peripheral action of the bioactive compound in the extract. This is consistent with findings of prior study (Mustafa et al. 2023) that observed significant reduction in BW after administration of similar doses of *E. ramosissimum* extracts.

The antidiabetic and nephron protective effect for *E. ramosissimum* observed in the present study can be explained by various phytochemicals, such as phenols and flavonoids, with pronounced antioxidant effects (Savaya et al. 2020). Of these, orientin, iso-orientin, quercetin, rutin, 7-Methoxy-4'-hydroxyflavone and succinic acid were all detected in the study extract. These compounds have been previously detected in other *Equisetum* species and shown antidiabetic and antioxidant effects in various models

(Vengerovskii et al. 2007; Zarubina et al. 2012; Rusmana et al. 2017; Malik et al. 2019; Ismail et al. 2020).

In addition, the HPLC analysis for the extract revealed the presence of ferulic acid isomers (isoferulic acid). This compound is well known to prevent the synthesis of advanced glycation end products, which is responsible for altering other proteins and generating oxidizing intermediates, causing oxidative stress in vascular cells and other tissues. As a result, this phytocomponent may play an essential preventive role in age-related illnesses and diabetic complications, including nephropathy (Meeprom et al. 2013). Moreover, the flavone derivative 7-Glu Chrysoeriol was detected in this study species for the first time. The latter was previously isolated from the leaves of *Ginkgo biloba* (Hua et al. 2013) and showed a protective effect against nephrotoxicity caused by diabetes in rats (Qiu et al. 2015). Similarly, Chrysoreriol derivatives were also detected in this study. It was previously isolated from *Cardiospermum halicacabum*, with a capacity to regulate glycemic management and upregulate carbohydrate metabolic enzymes in STZ-induced diabetic rats (Krishnan 2020).

The fatty acids, linolelaidic and myristic acids were observed in the prepared extracts. Compositions of fatty acids in four *Equisetum* sp. (*E. arvense*, *E. palustre*, *E. ramosissimum*, and *E. telmateia*) revealed that linoleic acid was the predominant fatty acid in these species. In contrast, myristic acid was the second major fatty acid (Kokten et al. 2020). The latter is considered an omega-6 trans fatty acid, whereas myristic acid poses an antioxidant effect (Liu et al. 2019). These fatty acids were shown to lower insulin-responsive to glucose levels and body weight. Therefore, it may be a promising candidate for managing diabetes and its complications (Takato et al. 2017). In addition, higher dietary linoleic acid intake was linked to a lower risk of diabetic complications (Dos Santos Alves et al. 2018).

## Conclusions

Accordingly, the antidiabetic and nephron protective effects observed in STZ-induced diabetic rats treated with the ethanolic extract of *E. ramosissimum* can be considered a novel finding. A significant reducing effect was observed on FBG, Cr, and U levels in STZ-induced diabetic rats.. This expectation was raised from the significant association between BW and FBG with UA and Cr levels in the STZ-diabetic rats treated with different doses of the extract.

The high content of phenols and flavonoids in the ethanol extracts of *E. ramosissimum* is likely to be responsible for the proposed effect via antioxidant activity. Therefore, further research is required to investigate its mechanism of action on the molecular level.

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