



eISSN 2284-0230 - pISSN 1826-883

<https://www.pagepressjournals.org/index.php/jbr/index>

Publisher's Disclaimer. E-publishing ahead of print is increasingly important for the rapid dissemination of science. The **Early Access** service lets users access peer-reviewed articles well before print / regular issue publication, significantly reducing the time it takes for critical findings to reach the research community.

These articles are searchable and citable by their DOI (Digital Object Identifier).

The **Journal of Biological Research** is, therefore, e-publishing PDF files of an early version of manuscripts that undergone a regular peer review and have been accepted for publication, but have not been through the typesetting, pagination and proofreading processes, which may lead to differences between this version and the final one.

The final version of the manuscript will then appear on a regular issue of the journal.

E-publishing of this PDF file has been approved by the authors.

J Biol Res 2026 [Online ahead of print]

To cite this Article:

Abdalla HO, Hussein RH. **Modulatory effects of *Portulaca oleracea* extract on blood glucose and oxidative stress in diabetic male rats.** *J Biol Res* doi: 10.4081/jbr.2026.15852

 ©The Author(s), 2026

Licensee [PAGEPress](#), Italy

Note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article.

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Submitted: 19 June 2026

Accepted: 1 September 2026

Early access: 15 September 2026

Modulatory effects of *Portulaca oleracea* extract on blood glucose and oxidative stress in diabetic male rats

Hiwa O. Abdalla, Ridha H. Hussein

Biology Department, College of Science, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq

Correspondence: Hiwa O. Abdalla, Biology Department, College of Science, University of Sulaimani, Sulaymaniyah, 46001, Kurdistan Region, Iraq. Tel.: +9647804444503. E-mail: hiwa.abdullah@univsul.edu.iq

Key words: diabetes, purslane, catalase, superoxide dismutase, glutathione.

Abstract

Impaired antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), with increased lipid peroxidation, contribute to diabetic complications. This study evaluated the effects of *Portulaca oleracea* ethanolic (70%) extract on blood glucose and oxidative stress markers in diabetic male rats. Twenty-four Sprague–Dawley rats were assigned to four groups (n = 6): diabetic control (DC), Diabetic + *Portulaca oleracea* extract (DPO) (250 mg/kg), Diabetic + metformin (DM) (100 mg/kg), and combined treatment (DMPO). By week 4 of treatment, DPO (median: 294 mg/dL, first quartile {Q1}- third quartile {Q3}: 210–366), DM (median: 125 mg/dL, Q1-Q3: 95–160), DMPO (median: 144 mg/dL, Q1-Q3: 116–280) showed significantly lower blood glucose levels than DC (median: 448 mg/dL, Q1-Q3: 419–600, $p < 0.05$). CAT levels increased significantly in DM (median 1.1 pg/mL, Q1-Q3: 0.9-1.4) and DMPO (1.1 pg/mL, Q1-Q3: 0.9-2.4) compared to DC (0.3 pg/mL, Q1-Q3: 0.2-0.6, $p = 0.001$, $p \leq 0.001$, respectively). DPO and DMPO had significantly decreased malondialdehyde (MDA) (21.3 ± 3.8 ng/mL; 22 ± 1.9 ng/mL, respectively) and increased SOD (19.1 ± 3.6 ng/mL; 23.5 ± 3.9 ng/mL, respectively) compared to DC (MDA: 29.3 ± 6.1 ng/mL, SOD: 11.9 ± 2.4 ng/mL; $p < 0.05$). *Portulaca oleracea* demonstrated antihyperglycemic and antioxidant effects.

Introduction

Diabetes mellitus, a major chronic metabolic disorder of the modern era, is defined as persistent hyperglycemia due to defective insulin secretion, insulin resistance, or both. According to the 11th edition of the International Diabetes Federation (IDF) Diabetes Atlas, the current estimate of adults aged 20–79 years living with diabetes worldwide is around 589 million, or about 1 in 9 adults globally, and is expected to reach 853 million by 2050.¹ In 2024, there were an estimated 3.4 million deaths attributed to diabetes or its complications, demonstrating the significant burden diabetes places on global health systems.¹ In addition to its classic vascular and neuropathic complications, diabetes mellitus has profound, often underappreciated effects on the endocrine and reproductive axis, and thus identification of effective therapeutic strategies is a research priority of great clinical urgency.² A well-established pathophysiologic link between diabetes and systemic organ damage is the induction of oxidative stress. In diabetes, oxidative stress is due to an imbalance between oxidant and antioxidant systems, resulting from the increased generation of free radicals, coupled with the reduced activity of endogenous antioxidant enzymes, nonenzymatic glycosylation of proteins, glucose auto-oxidation, impaired glutathione metabolism, and increased lipid peroxide formation.³ Malondialdehyde (MDA), the final product of lipid peroxidation, is a reliable marker for oxidative tissue damage, while the enzymatic antioxidants, Superoxide Dismutase (SOD), Catalase (CAT), and reduced Glutathione (GSH), are the main cellular defenses against Reactive Oxygen Species (ROS).^{3,4} In diabetic conditions, the inhibition of these enzymatic pathways leads to cellular damage in several tissues, including gonads and accessory reproductive organs.^{2,5,6} Animal model studies have shown that Streptozotocin (STZ)-induced diabetes significantly decreases antioxidant enzyme activity and levels, consistent with clinical observations in diabetic human populations.⁷ These converging lines of evidence underscore the need for an urgent investigation of therapeutic agents that can simultaneously target hyperglycemia, oxidative stress, and hormonal derangement in diabetic patients. Metformin (biguanide) is the first-line pharmacological treatment for type 2 diabetes worldwide, which mainly acts by increasing peripheral insulin sensitivity and reducing hepatic gluconeogenesis.⁴ But metformin monotherapy does not completely solve the oxidative and hormonal complications related to diabetes.⁸ *Portulaca oleracea* L. (purslane) is a succulent annual herb belonging to the family Portulacaceae and has been of considerable scientific interest because of its wide pharmacological profile. According to the World Health Organization (WHO), purslane is one of the most widely used medicinal plants throughout the world and is an abundant source of antioxidants, such as vitamins A, C, and E, β -carotene, and glutathione, as well as omega-3 fatty acids, flavonoids, coumarins, alkaloids, saponins, polyphenols, and anthocyanins.⁹ Its aqueous extract presented no cytotoxicity or genotoxicity, and it has been approved for daily consumption.⁹ It has been experimentally proven to have anti-diabetic, antioxidant, anti-inflammatory, and anti-hyperlipidemic effects in several *in vivo* models.^{3,4,10,11} More recently, the combination of *P. oleracea* ethanolic extract with metformin has been evaluated for hepatoprotective effects in diabetic rats with complementary beneficial effects on oxidative and inflammatory markers.⁴ Despite this growing body of evidence, several important gaps remain in the literature. The present study was designed to evaluate the effects of *Portulaca oleracea* extract on

blood glucose, MDA, SOD, CAT, and GSH in STZ-induced diabetic male rats in four experimental groups.

Materials and Methods

Ethical approval

All procedures complied with the ARRIVE guidelines, Basel Declaration, and the 3Rs principles (Replacement, Reduction, and Refinement).^{12,13} Ethical approval was obtained from the Ethical Research Committee of the College of Science, University of Sulaimani (Approval No. 6, dated 24/8/2025). Efforts were made to minimize suffering in animals and the number of animals used.

Preparation of *Portulaca oleracea* extract

Plant collection and preparation

The *Portulaca oleracea* plant material (fresh whole plant) used in the present study was collected from Sulaymaniyah Governorate, Kurdistan Region, Iraq, during the summer of 2025. The plant was morphologically authenticated by specialists at the College of Agricultural Engineering Sciences, University of Sulaimani. Plant material was washed with tap and distilled water, shade-dried at room temperature for 3 weeks, ground into powder, sieved (40-mesh), and stored in airtight amber containers until extraction.

Ethanol extraction and dose preparation

Dried powder was extracted by cold maceration using 70% ethanol at a 1:10 (w/v) ratio for 72 h at room temperature with intermittent stirring. The mixture was filtered through muslin cloth followed by Whatman No.1 filter paper. Filtrates were evaporated at room temperature for 14 days until constant weight was achieved. Extraction yield was 11.58%, and the dried extract was stored at -20°C in amber vials. High-Performance Liquid Chromatography (HPLC)/gas chromatography-mass spectrometry analysis was performed to identify major phenolic, flavonoid, and fatty acid constituents (Table 1). Fresh extract solutions were prepared daily in distilled water. The total amount of extract used over the 30-day experimental period was approximately 45 g.

Animals and experimental design

Twenty-four male Sprague–Dawley rats (200–300 g, 8 weeks old) were obtained from the animal house of the Biology Department, University of Sulaimani. Animals were housed under standard conditions ($24 \pm 2^{\circ}\text{C}$, 12 h light/dark cycle) with free access to food and water and acclimatized for 7 days before experimentation. Rats were randomly (simple randomization) divided into four groups ($n =$

6): i) Diabetic Control (DC): received only distilled water; ii) Diabetic + *Portulaca oleracea* (DPO): received only PO extract (250 mg/kg); iii) Diabetic + Metformin (DM): received only metformin (100 mg/kg); iv) Diabetic + Metformin + PO (DMPO): received both metformin (100 mg/kg) + PO extract (250 mg/kg). All treatments were administered orally by gavage once daily for 4 weeks after dissolving the extract and metformin in sterile distilled water.

The dose of 250 mg/kg body weight of *Portulaca oleracea* extract was selected based on previous experimental studies demonstrating significant antihyperglycemic and antioxidant efficacy in diabetic rodent models while remaining within a well-tolerated dose range.^{3,7,14} This intermediate dose was chosen to provide therapeutic efficacy while avoiding unnecessary administration of higher doses, consistent with the ARRIVE 2.0 guidelines and the 3Rs principles.^{12,13}

Induction of diabetes mellitus

Experimental diabetes was induced by intraperitoneal injection of streptozotocin (STZ; 50 mg/kg) freshly dissolved in cold 0.1 M citrate buffer (pH 4.5). Rats were fasted for 12 h before injection. Following injection, rats received 10% sucrose solution for 48 h to prevent hypoglycemia. After 72 h, fasting blood glucose was measured with a glucometer (On Call Plus, ACON Laboratories, Inc., San Diego, California, USA). Rats with glucose levels ≥ 200 mg/dL were diagnosed with diabetes. This threshold is commonly accepted in the literature as an indicator of successful STZ-induced diabetes in rodent models.¹⁵ The same glucometer was used to check blood glucose weekly during the study.

Sample collection and biochemical analysis

Rats were fasted overnight at the completion of the experiment and anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg). Blood was collected by cardiac puncture, allowed to clot, and centrifuged at 3,000 rpm for 10–15 min at 4°C. Serum samples were stored at –80°C until analysis. Serum levels of MDA, SOD, CAT, and GSH were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (SUNLONG Biotech Co. Ltd, Hangzhou, Zhejiang Province, China), according to the manufacturer's instructions. All assays were performed in duplicate, and the mean was calculated. The details of sample kits for parameters were as follows: CAT (Sensitivity: 0.25 pg/mL; Standard: 54 pg/mL; Assay range: 1 pg/mL - 36 pg/mL), MDA (Assay range: 6 ng/mL-350 ng/mL; Standard: 450 ng/mL; Sensitivity: 1.2 ng/mL), GSH (Assay range: 18 ng/L-500 ng/L; Sensitivity: 3.5 ng/L; Standard: 720 ng/L), SOD (Sensitivity: 0.6 ng/mL; Assay range: 3 ng/mL-200 ng/mL; Standard: 270 ng/mL).

Statistical analysis

Data were initially collected using Microsoft Excel and subsequently encoded and analyzed using Statistical Package for the Social Sciences (SPSS) Statistics (version 27) (IBM Co., Armonk, New

York, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Normally distributed variables were expressed as mean $\pm$ standard deviation, whereas non-normally distributed variables were presented as median and Interquartile Range (IQR). When blood glucose data were assessed collectively across all four groups and study weeks, the data showed an approximately normal distribution. However, following stratification by individual group and study week, the data did not consistently meet the normality assumption. Therefore, descriptive statistics and subsequent analyses were selected according to the distribution of the data at the respective level of analysis. For comparisons among the groups, the independent-samples Kruskal–Wallis test was used for non-normally distributed variables, including blood glucose and CAT. The parametric variables, GSH, MDA, and SOD, were analyzed by one-way Analysis of Variance (ANOVA). Levene’s test was used to assess homogeneity of variances to decide on the post hoc analysis after ANOVA. Tukey’s post hoc test was used when the assumption of equal variances was met, and the Games–Howell post hoc test was used when the assumption of homogeneity of variances was violated. Simple boxplots were also drawn for variables analyzed with the Kruskal–Wallis test to assess distribution patterns. The level of significance was set at $p < 0.05$.

Results

Descriptive analysis of the overall blood glucose levels showed a progressive decrease within the study from Week 1 (444.33 ± 87.38 mg/dL), Week 2 (374.83 ± 109.12 mg/dL), Week 3 (319.83 ± 108.68 mg/dL) to Week 4 (274.46 ± 159.92 mg/dL). The descriptive data of blood glucose (within weeks and groups) and oxidative parameters are summarized in Table 2. Kruskal–Wallis test revealed no statistically significant differences in blood glucose levels between the four groups in Week 1 ($\chi^2(3) = 7.380$, $p = 0.06$) (Figure 1A). In Week 2, no significant differences were found between the groups ($\chi^2(3) = 5.669$, $p = 0.13$) (Figure 1B). Meanwhile, a statistically significant difference was found in Week 3 ($\chi^2(3) = 10.724$, $p = 0.01$) (Figure 1C). Post hoc pairwise comparisons showed a significant difference between the DM and DC groups (standardized test statistics = 3.1, $p = 0.002$). Furthermore, there was a significant reduction in blood glucose in the DM group compared to DPO (standardized test statistics = -2.4, $p = 0.01$). The greatest between-group difference was observed in Week 4, where the Kruskal–Wallis test indicated a highly significant effect ($\chi^2(3) = 16.101$, $p = 0.001$) (Figure 1D). The pairwise comparisons identified three significant differences: DC vs DM (standardized test statistics = 3.7, $p < 0.001$), DC vs DMPO (standardized test statistics = 3.1, $p = 0.002$), and DC vs DPO (standardized test statistics = 2.0, $p = 0.04$). All three treatment groups had significantly lower blood glucose levels than the DC group at the end of the study period. The DM and DMPO groups showed a greater reduction in blood glucose compared with DPO (standardized test statistics = -1.7 and -1.1, respectively), but the difference was not statistically significant (Table 3).

Independent-Samples Kruskal–Wallis test showed statistically significant differences in CAT levels between the groups ($H = 16.525$, $df = 3$, $p = 0.001$). Pairwise comparisons showed significant differences between the DC and DM groups ($p = 0.001$), between the DC and DMPO groups ($p < 0.001$), and between the DPO and DMPO groups ($p = 0.04$). However, there were differences between

the DC and DPO groups, but not at the significance level ($p = 0.11$). There were also no statistically significant differences between the DPO and DM groups ($p = 0.09$), or the DM and DMPO groups ($p = 0.76$) (Table 4).

The levels of GSH, MDA and SOD in the four study groups were compared using one-way ANOVA. There was no statistically significant difference in GSH levels among the groups ($F(3,20) = 2.782$, $p = 0.06$). There was a significant difference in MDA levels among the groups ($F(3,20) = 5.320$, $p = 0.007$). Post hoc analysis found that the MDA level was significantly higher in the DC group than in the DPO group (mean difference - MD = 8.00, $p = 0.01$) and in the DMPO group (MD = 7.32, $p = 0.02$). No statistically significant differences were observed between the remaining group comparisons (all $p > 0.05$). SOD levels differed significantly among the study groups ($F(3,20) = 7.215$, $p = 0.002$). Pairwise comparisons revealed significantly lower SOD levels in the DC group compared with the DPO group (MD = -7.20, $p = 0.01$) and the DMPO group (MD = -11.60, $p = 0.001$). No statistically significant differences were identified between the remaining group comparisons ($p > 0.05$) (Table 5).

Discussion

The present study demonstrated that blood glucose levels in both the DM and DPO groups did not differ significantly during the first and second weeks of treatment. This finding indicates that the antihyperglycemic effects of both interventions were not immediate and may require prolonged administration before becoming metabolically evident. A similar time-dependent glucose-lowering response was also reported by Samarghandian *et al.*, where significant reductions in blood glucose in STZ-induced diabetic rats treated with *P. oleracea* aqueous extract were slightly more prominent after several weeks of continuous treatment, implying that the antihyperglycemic activity of the plant may develop gradually over time due to cumulative biological effects on glucose metabolism and oxidative stress pathways.³ Ramadan *et al.* also showed that the remarkable improvements in hyperglycemia and pancreatic histopathology in diabetic rats treated with purslane aqueous extract were more pronounced after sustained treatment periods and not during the early phase of intervention.¹⁴

The metformin-treated group showed significantly lower blood glucose levels than the diabetic control group at the third week of treatment. This is consistent with the known pharmacology of metformin, which has been shown to reduce hyperglycemia by inhibiting hepatic gluconeogenesis, increasing peripheral glucose uptake and improving insulin sensitivity.¹⁶ The earlier onset of antihyperglycemic effect of metformin as compared to *P. oleracea* in the present study may be due to the direct pharmacodynamic action of metformin, while herbal interventions may require longer duration of exposure to produce measurable metabolic and antioxidant effects.

The significant reduction in blood glucose level in *P. oleracea* treated groups at the 4th week confirms the antidiabetic potential of ethanolic extract in STZ-induced diabetes. This result is in line with the findings of Ramadan *et al.*, who reported that administration of *P. oleracea* aqueous extract significantly reduced the fasting blood glucose level and improved the pancreatic tissue architecture in diabetic rats.¹⁴ Also, Bai *et al.* have shown that polysaccharides isolated from *P. oleracea* ethanolic

extract significantly lowered the blood glucose levels and improved insulin resistance in diabetic rats. These results suggested that bioactive compounds of purslane have a significant role in the regulation of glucose.¹⁷ El-Sayed also reported similar findings of significant antihyperglycemic activity of purslane supplementation in diabetic subjects, attributing these effects to the plant's rich antioxidant and phytochemical composition.¹⁸

The delayed but remarkable antihyperglycemic effect in the present study may be attributed to the antioxidant and pancreatic protective properties of *P. oleracea*. It has been previously shown that *P. oleracea* contains flavonoids, alkaloids, omega-3 fatty acids, phenolic compounds, and polysaccharides with strong antioxidant activity and levels.¹⁹ Samarghandian *et al.* showed that treatment with *P. oleracea* aqueous extract significantly reduced MDA levels and increased GSH and total antioxidant capacity in diabetic rats, which may be associated with the amelioration of oxidative stress and improvement in glycemic control and pancreatic function.³ Moreover, recently Aziz *et al.* showed that *P. oleracea* methanolic extracts attenuated diabetic complications via modulation of oxidative stress pathways, activation of Nrf2-mediated antioxidant defenses, and inhibition of inflammatory signaling pathways.²⁰ Taken together, these results indicate that the glucose-lowering effects observed in the present study may be secondary to progressive improvement of oxidative balance and preservation of remaining pancreatic β -cells.

Some studies reported earlier or more pronounced antihyperglycemic effects after administration of *P. oleracea*, contrary to our findings. The significant decreases of blood glucose at earlier stages of treatment in the higher doses of purslane extract suggest that the time of onset and magnitude of the antihyperglycemic effect might be dose-dependent.³ Differences among studies could be associated with variations in extraction methods, phytochemical constituents, treatment period, induction severity of diabetes and animal models used in experiments. In a previous study, it was shown that ethanolic extracts contain higher levels of phenolic and lipophilic antioxidant compounds than aqueous extracts, which could have an effect on biological efficacy.²¹

In the present study, marked alterations in the oxidative stress biomarkers were observed in experimental groups. This study validated the antioxidant effect of *Portulaca oleracea* in STZ-induced diabetic rats. It is well known that streptozotocin-induced diabetes results in excessive generation of ROS that causes oxidative damage to cellular macromolecules and depletion of endogenous antioxidant defenses. Oxidative stress has been recognized as an important factor contributing to β -cell dysfunction, insulin resistance and progression of diabetic complications.^{22,23}

CAT levels were different among the study groups, with higher levels in the DM, DPO, and DMPO than in the DC group. These results indicate that both metformin and *P. oleracea* improved the antioxidant defense mechanisms that were compromised by STZ-induced oxidative stress. Similar results were reported by Samarghandian *et al.*, who demonstrated that administration of *P. oleracea* significantly improved antioxidant status in diabetic rats by enhancing endogenous antioxidant enzyme activities and reducing oxidative damage.³ Likewise, other studies have shown that administration of purslane restored the activities of antioxidant enzymes and reduced oxidative injury in diabetic animals, suggesting that the plant has protective effects by modulating oxidative stress pathways.¹⁴ The increase

in CAT level observed in the present study may be attributed to the high content of flavonoids, phenolic compounds, vitamins, and omega-3 fatty acids in *P. oleracea*.

The results of CAT level showed no statistically significant differences between DPO and DM and between DM and DMPO groups, suggesting that *P. oleracea* alone may have antioxidant effects comparable to those of metformin. Similar observations have been reported in experimental diabetes studies where extracts of botanicals rich in antioxidants increased the activities of antioxidant enzymes to the same level of conventional antidiabetic therapies.¹⁹ However, the significantly higher CAT level in DMPO group compared with DPO group might indicate an additive antioxidant effect of purslane in combination with metformin, although this was not consistently shown in all statistical comparisons.

Unlike CAT, GSH levels were not significantly different among the groups of the study. This result indicates that even though the interventions upregulated some of the antioxidant defense systems, their effect on GSH homeostasis was not enough to cause statistically detectable alterations during the period of the study. Hence, this finding may have been influenced by limited statistical power, rather than indicating a true absence of an effect. In addition, some investigators have reported similar findings. Nazaroglu *et al.* found that treatment with glyburide in streptozotocin-induced diabetic rats restored SOD and CAT levels, whereas glutathione peroxidase did not show a similar significant response. These findings suggest that antioxidant interventions may differentially affect components of the endogenous antioxidant defense system.²⁴ The lack of major changes in GSH may also be related to the short duration of treatment or to the extent of oxidative damage induced by STZ that may require longer time periods to recover before the intracellular GSH stores are replenished. A study that evaluated antioxidant interventions in diabetic models has also reported that GSH recovery was partial in comparison to restoration of antioxidant enzyme activities.²⁵

MDA, a well-established indicator of lipid peroxidation and oxidative tissue damage, was significantly increased in the diabetic control group compared with the DPO and DMPO groups. The results indicate that *P. oleracea* treatment significantly reduced oxidative damage associated with diabetes. Other authors have reported similar reductions in MDA levels after administration of *P. oleracea* and proved that purslane administration significantly attenuated lipid peroxidation in diabetic rats.³ In agreement, Aziz *et al.* showed that methanolic extracts of *P. oleracea* reduced oxidative tissue damage and inhibited markers of lipid peroxidation by activation of antioxidant defense pathways.²⁰ The decrease of MDA in the present study has biological significance since increased lipid peroxidation is involved in membrane damage, β -cell dysfunction, and the pathogenesis of diabetic complications.^{23,26}

Furthermore, SOD level was significantly increased in the DPO and DMPO groups compared with the diabetic control group, which further supports the antioxidant effect of *P. oleracea*. SOD is the first enzymatic line of defense against oxidative stress, catalyzing the conversion of superoxide radicals to hydrogen peroxide, which is detoxified by CAT.²⁷ Similar increases in SOD level after administration of purslane have been reported in diabetic animals²⁸ where improvements in antioxidant enzyme levels were accompanied by decreases in oxidative damage and improved metabolic outcomes.³ In the present study, the simultaneous increase of SOD and CAT activities suggests a co-ordinate upregulation of

endogenous antioxidant defenses and may have contributed to the reduction of blood glucose levels during the later stages of treatment.

However, uniform improvement in antioxidant biomarkers following *P. oleracea* administration was not observed in all studies. Some studies showed significant reductions in lipid peroxidation without any increase in all antioxidant enzymes, whereas other studies reported dose-dependent responses which were different depending on the extraction method, duration of treatment and severity of disease.²⁹ Such discrepancies could be attributed to the difference in the phytochemical composition of the extracts, as well as the difference in the experimental design and biomarker assessment. But the overall trend of the present study such as increased activities of CAT and SOD and decreased levels of MDA strongly supports the notion that *P. oleracea* ameliorates diabetes associated oxidative stress. These antioxidant effects might have contributed to the improved glycemic control in the treated groups by protecting residual pancreatic tissues from oxidative injury and preserving cellular antioxidant capacity. The results indicate that *P. oleracea* possesses significant antihyperglycemic activity and the similar reduction of glycemic levels in the combined-treatment group might be a reflection of the complementary mechanisms of metformin and *P. oleracea*, especially through concurrent enhancement of oxidative stress attenuation. The present findings together support the potential therapeutic role of *P. oleracea* as a natural antihyperglycemic and antioxidant agent in experimental diabetes. Several limitations should be considered when interpreting the findings of this study. The small sample size, short duration of treatment and assessment of only one dose of *P. oleracea* may have hindered the ability to detect dose-dependent and long-term effects. Moreover, the study was limited to male rats and a streptozotocin-induced diabetic model, which may restrict the extrapolation of the findings. The assessment was confined to a few biochemical markers and no histopathological and mechanistic studies were conducted to further elucidate the biological effects of the treatment. In addition, the specific bioactive compounds responsible for the antihyperglycemic and antioxidant effects observed were not individually investigated, although the extract was characterized by HPLC/gas chromatography-mass spectrometry. As the HPLC/gas chromatography-mass spectrometry analysis was outsourced to an external laboratory and was not conducted in our facility, we were unable to provide the detailed HPLC analytical procedure. Finally, although the plant was morphologically authenticated by specialists, a formal herbarium voucher specimen was not deposited.

Conclusions

Portulaca oleracea possesses both antihyperglycemic and antioxidant activities in experimental diabetes. Although its glucose-lowering effect was less pronounced and developed more slowly than that of metformin, PO treatment significantly reduced blood glucose levels and improved oxidative stress status through increasing CAT and SOD levels and decreasing lipid peroxidation. The absence of changes in GSH levels suggests that the antioxidant effects of PO are selective rather than universal across all antioxidant pathways. Moreover, PO supplementation to metformin did not confer significant advantages over metformin alone, indicating that PO may be more beneficial as a complementary antioxidant intervention than as a potentiator of metformin efficacy.

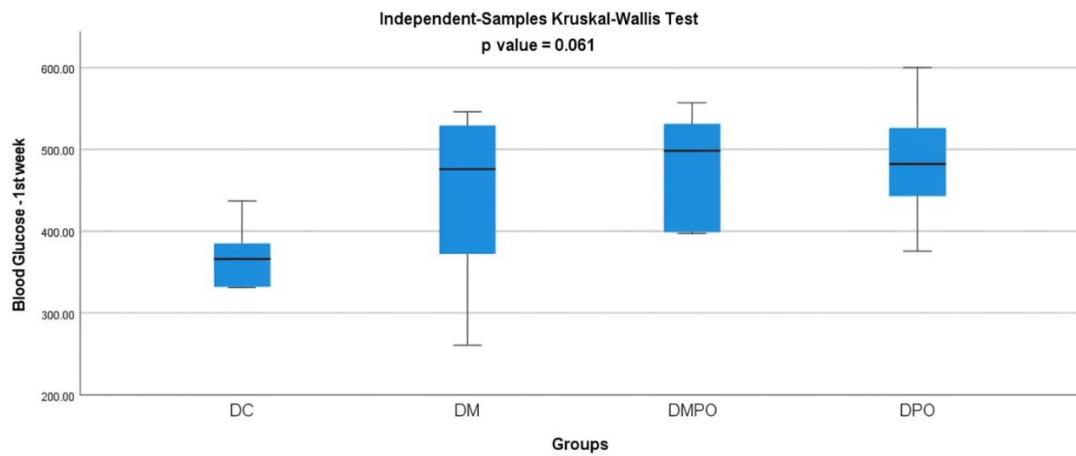
References

1. Genitsaridi I, Salpea P, Salim A, et al. 11th edition of the IDF Diabetes Atlas: global, regional, and national diabetes prevalence estimates for 2024 and projections for 2050. *Lancet Diabetes Endocrinol* 2026;14:149-56.
2. Migdalis IN. Chronic complications of diabetes: Prevalence, prevention, and management. *J Clin Med* 2024;13:7001.
3. Samarghandian S, Borji A, Farkhondeh T. Attenuation of oxidative stress and inflammation by *Portulaca oleracea* in streptozotocin-induced diabetic rats. *J Evid-Based Complement Altern Med* 2017;22:562-66.
4. Bahr N, Nour AHM, El-Kader AMA, et al. Hepatic restorative potential of *Portulaca oleracea* ethanolic extract and/or metformin in type II diabetes mellitus model induced by high-fat diet and STZ. *J Basic Appl Zool* 2025;86:62.
5. Luo Z, Wang L, Luo J, et al. Antidiabetic agents and male fertility: unraveling the impact of glucose-lowering therapies on sperm quality in diabetic males—a narrative review. *Transl Androl Urol* 2025;14:3387-3401.
6. Dhindsa S, Ghanim H, Batra M, Dandona P. Hypogonadotropic hypogonadism in men with diabetes. *Diabetes Care* 2018;41:1516-1525.
7. Rakhshandeh H, Rajabi Khasevan H, Saviano A, et al. Protective effect of *Portulaca oleracea* on streptozotocin-induced type I diabetes-associated reproductive system dysfunction and inflammation. *Molecules* 2022;27:6075.
8. Martin Martins J, Pina Jorge MD, Martins Maia C, et al. Primary and secondary hypogonadism in male persons with diabetes mellitus. *Int J Endocrinol* 2021;2021:1-9.
9. Fatemi Tabatabaei SR, Rashno M, Ghaderi S, Askaripour M. The aqueous extract of *Portulaca oleracea* ameliorates neurobehavioral dysfunction and hyperglycemia related to streptozotocin-diabetes induced in ovariectomized rats. *Iran J Pharm Res IJPR* 2016;15:561-71.
10. Saad EA, Hassan HA, Ghoneum MH, Alaa El-Dein M. Edible wild plants, chicory and purslane, alleviated diabetic testicular dysfunction, and insulin resistance via suppression 8OHdg and oxidative stress in rats. *PloS One* 2024;19:e0301454.

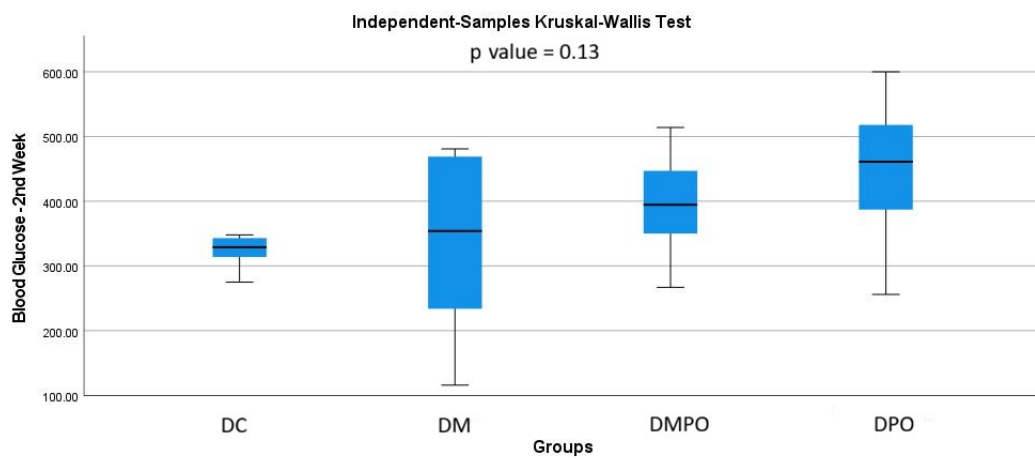
11. Miao L, Zhou C, Zhang H, et al. *Portulaca oleracea* L. (purslane) extract protects endothelial function by reducing endoplasmic reticulum stress and oxidative stress through AMPK activation in diabetic obese mice. *Antioxidants* 2023;12:2132.
12. Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. *Animals* 2019;9:754.
13. Percie Du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *J Cereb Blood Flow Metab.* 2020;40:1769-77.
14. Ramadan BK, Schaalán MF, Tolba AM. Hypoglycemic and pancreatic protective effects of *Portulaca oleracea* extract in alloxan induced diabetic rats. *BMC Complement Altern Med* 2017;17:37.
15. Rehman HU, Ullah K, Rasool A, et al. Comparative impact of streptozotocin on altering normal glucose homeostasis in diabetic rats compared to normoglycemic rats. *Sci Rep* 2023;13:7921.
16. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia* 2017;60:1577-1585.
17. Bai Y, Zang X, Ma J, Xu G. Anti-diabetic effect of *Portulaca oleracea* L. polysaccharide and its mechanism in diabetic rats. *Int J Mol Sci* 2016;17:1201.
18. El-Sayed MIK. Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *J Ethnopharmacol* 2011;137:643-51.
19. Zhou YX, Xin HL, Rahman K, et al. *Portulaca oleracea* L.: a review of phytochemistry and pharmacological effects. *BioMed Res Int* 2015;2015:925631.
20. Aziz WM, Ahmed SA, Shaker SE, et al. *Portulaca oleracea* L seed extracts counteract diabetic nephropathy through SDF-1/IL10/PPAR γ –mediated tuning of keap1/Nrf2 and NF- κ B transcription in Sprague Dawley rats. *Diabetol Metab Syndr* 2024;16:119.
21. Lee JE, Jayakody J, Kim JI, et al. The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. *Foods* 2024;13:3151.
22. Nahdi AMTA, John A, Raza H. Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in rin-5f pancreatic β -cells. *Oxid Med Cell Longev* 2017;2017:7054272.
23. Eguchi N, Vaziri ND, Dafoe DC, Ichii H. The role of oxidative stress in pancreatic β cell dysfunction in diabetes. *Int J Mol Sci* 2021;22:1509.

24. Nazaroglu NK, Sepici-Dincel A, Altan N. The effects of sulfonylurea glyburide on superoxide dismutase, catalase, and glutathione peroxidase activities in the brain tissue of streptozotocin-induced diabetic rat. *J Diabetes Complications* 2009;23:209-13.
25. Sadi G, Eryilmaz N, Tütüncüoğlu E, et al. Changes in expression profiles of antioxidant enzymes in diabetic rat kidneys. *Diabetes Metab Res Rev* 2012;28:228-35.
26. Shabalala SC, Johnson R, Basson AK, et al. Detrimental effects of lipid peroxidation in type 2 diabetes: exploring the neutralizing influence of antioxidants. *Antioxidants* 2022;11:2071.
27. He L, He T, Farrar S, et al. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell Physiol Biochem* 2017;44:532-53.
28. Sharma A, Vijayakumar M, Rao ChV, et al. Action of *Portulaca oleracea* against streptozotocin-induced oxidative stress in experimental diabetic rats. *J Complement Integr Med* 2009;6:12-18.
29. Ghorani V, Saadat S, Khazdair MR, et al. Phytochemical characteristics and anti-inflammatory, immunoregulatory, and antioxidant effects of *Portulaca oleracea* L.: A comprehensive review. Novaes RD, ed. *Evid Based Complement Alternat Med* 2023;2023:2075444.

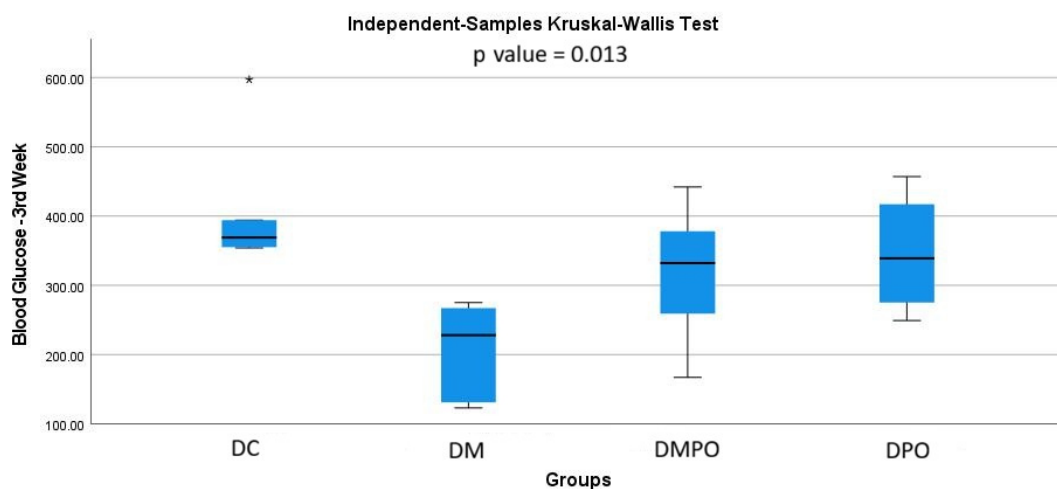
Figure 1. Independent-samples Kruskal-Wallis test showing the blood glucose levels in each group at the first week (A), second week (B), third week (C), and fourth week (D). Boxplots showing blood glucose levels across the experimental groups during weeks 1–4. The boxes represent the interquartile range – IQR (25th–75th percentiles), the horizontal lines within the boxes indicate the median, and the whiskers represent the most extreme non-outlying observations (within $1.5 \times \text{IQR}$). The asterisk (*) indicates an extreme outlier ($>3 \times \text{IQR}$). Each group comprised six rats ($n = 6$). The p values shown above the plots were obtained using the independent-samples Kruskal–Wallis test.



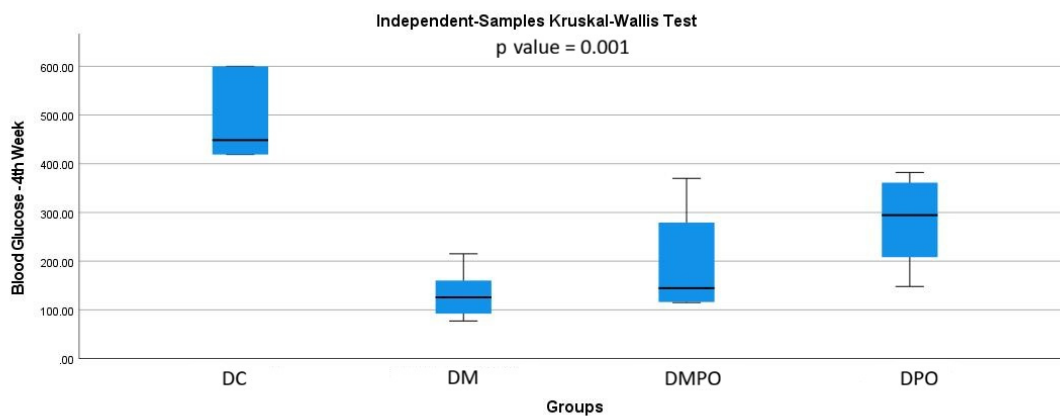
A



B



C



D

Table 1. HPLC/GC chromatography analysis of the *Portulaca oleracea* extract used in the study.

Compound	Standard		Sample	
	Peak area	Conc. (mg/L)	Peak area	Conc. (mg/L)
Gallic acid	5178.598	5.0	22523.709	21.7
Luteolin	849.227	5.0	3396.908	20.0
Rutin	16954.340	20.0	23736.076	28.0

Compound	Standard		Sample	
	Peak area	Conc. (mg/L)	Peak area	Conc. (mg/L)
Kaempferol	1248.963	10.0	1124.066	9.0
Vanillic acid	231.308	10.0	254.438	11.0
Caffeic acid	1716.254	10.0	6521.765	38.0
Quercetin	945.576	5.0	2836.728	15.0
p-Coumaric acid	5909.551	25.0	3782.112	16.0
Apigenin	11434.438	10.0	10290.994	9.0
Ferulic acid	1570.625	10.0	3455.377	22.0
Chlorogenic acid	922.277	20.0	2213.465	48.0
Catechin	582.900	100.0	204.015	35.0
Palmitic acid*	483.860	10.0	2709.616	56.0
Stearic acid*	376.201	10.0	3310.568	88.0
Oleic acid*	333.737	10.0	3103.754	93.0
Linoleic acid (C18:2 n-6)*	1502.905	100.0	3071.084	204.3
Linolenic acid (C18:3 n-3)*	776.080	10.0	24368.912	314.0

*Concentration analysis was performed by GC chromatography

GC, Gas Chromatography; HPLC, High-Performance Liquid Chromatography; Conc., Concentration. Sample concentrations were calculated by single-point external calibration, $C_{\text{sample}} = (A_{\text{sample}} / A_{\text{standard}}) \times C_{\text{standard}}$, and refer to the injected extract solution (mg/L).

Table 2. Descriptive data on blood glucose across the study period and oxidative parameters

Groups	Blood Glucose (mg/dL)			
	1 st week (median, Q1-Q3)	2 nd week (median, Q1-Q3)	3 rd week (median, Q1-Q3)	4 th week (median, Q1-Q3)
DC	366 (331- 384)	329 (304 - 344)	369 (354 - 395)	448 (419 - 600)

DPO	482 (444 - 526)	461 (390 - 520)	339 (268 - 427)	294 (210 - 366)
DM	475 (374 - 533)	354 (235 - 472)	228 (129 - 269)	125 (95 - 160)
DMPO	498 (398 - 537)	394 (350 - 450)	332 (265 - 380)	144 (116 - 280)
	CAT (pg/mL) (median, Q1-Q3)			
DC	0.3 (0.2 – 0.6)			
DPO	0.8 (0.7 – 0.9)			
DM	1.1 (0.9 – 1.4)			
DMPO	1.1 (0.9 – 2.4)			
	MDA (ng/mL) (mean $\pm$ SD)			
DC	29.3 $\pm$ 6.1			
DPO	21.3 $\pm$ 3.8			
DM	25.6 $\pm$ 2.3			
DMPO	22 $\pm$ 1.9			
	SOD (ng/mL) (mean $\pm$ SD)			
DC	11.9 $\pm$ 2.4			
DPO	19.1 $\pm$ 3.6			
DM	25.3 $\pm$ 9.2			
DMPO	23.5 $\pm$ 3.9			
	GSH (ng/L) (mean $\pm$ SD)			
DC	21 $\pm$ 11.9			
DPO	35 $\pm$ 12.7			
DM	37.3 $\pm$ 11.6			
DMPO	35.6 $\pm$ 8.3			

DC, diabetic control; DPO, diabetic group receiving portulaca oleracea extract; DM, diabetic group receiving metformin; DMPO, diabetic group receiving both metformin and portulaca oleracea; Q1, first quartile; Q3, third quartile; CAT, catalase; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; SD, standard deviation

Table 3. Pairwise comparisons of the groups regarding blood glucose in the 3rd and 4th weeks.

Blood Glucose in 3 rd week				
	Test Statistics	Standard Error	Standardized Test Statistics	p value
DM vs DMPO	-7.7	4.1	-1.9	0.058
DM vs DPO	-9.8	4.1	-2.4	0.016

DC vs DM	12.7	4.1	3.1	0.002
DMPO vs DPO	-2.1	4.1	-0.5	0.610
DC vs DMPO	5.0	4.1	1.2	0.220
DC vs DPO	2.9	4.1	0.7	0.475
Blood Glucose in 4th week				
DM vs DMPO	-2.8	4.1	-0.7	0.487
DM vs DPO	-7.1	4.1	-1.7	0.079
DC vs DM	15.3	4.1	3.7	<0.001
DMPO vs DPO	-4.3	4.1	-1.1	0.288
DC vs DMPO	12.5	4.1	3.1	0.002
DC vs DPO	8.1	4.1	2.0	0.045

DM, diabetic group received only metformin; DMPO, diabetic group received combination of metformin and the extract; DPO, diabetic group received the extract only; DC, diabetic control.

Table 4. Pairwise comparisons of model groups regarding catalase level based on the Kruskal-Wallis test.

Catalase				
Groups	Test Statistics	Std. Error	Std. Test Statistics	p value
DC vs DPO	-6.41	4.07	-1.57	0.11
DC vs DM	-13.33	4.07	-3.27	0.001
DC vs DMPO	-14.58	4.07	-3.58	<0.001
DPO vs DM	-6.91	4.07	-1.69	0.09
DPO vs DMPO	-8.16	4.07	-2.00	0.04
DM vs DMPO	1.25	4.07	0.30	0.76

DM, diabetic group received only metformin; DMPO, diabetic group received combination of metformin and the extract; DPO, diabetic group received the extract only; DC, diabetic control.

Table 5. Multiple comparisons of GSH, SOD, and MDA levels among the model groups.

Dependent Variable	(I) Groups	(J) Groups	MD (I-J)	Std. Error	p value	95% Confidence Interval	
						LB	UB
Glutathione level	DC	DPO	-14.21667	6.51016	.162	-32.4382	4.0049
		DMPO	-14.88333	6.51016	.135	-33.1049	3.3382
		DM	-16.58333	6.51016	.083	-34.8049	1.6382
	DPO	DC	14.21667	6.51016	.162	-4.0049	32.4382
		DMPO	-.66667	6.51016	1.000	-18.8882	17.5549
		DM	-2.36667	6.51016	.983	-20.5882	15.8549
	DMPO	DC	14.88333	6.51016	.135	-3.3382	33.1049
		DPO	.66667	6.51016	1.000	-17.5549	18.8882
		DM	-1.70000	6.51016	.994	-19.9215	16.5215
	DM	DC	16.58333	6.51016	.083	-1.6382	34.8049
		DPO	2.36667	6.51016	.983	-15.8549	20.5882
		DMPO	1.70000	6.51016	.994	-16.5215	19.9215
MDA level	DC	DPO	8.00000*	2.26062	.010	1.6727	14.3273
		DMPO	7.31667*	2.26062	.020	.9893	13.6440
		DM	3.71667	2.26062	.378	-2.6107	10.0440
	DPO	DC	-8.00000*	2.26062	.010	-14.3273	-1.6727
		DMPO	-.68333	2.26062	.990	-7.0107	5.6440
		DM	-4.28333	2.26062	.262	-10.6107	2.0440
	DMPO	DC	-7.31667*	2.26062	.020	-13.6440	-.9893
		DPO	.68333	2.26062	.990	-5.6440	7.0107
		DM	-3.60000	2.26062	.405	-9.9273	2.7273
	DM	DC	-3.71667	2.26062	.378	-10.0440	2.6107
		DPO	4.28333	2.26062	.262	-2.0440	10.6107
		DMPO	3.60000	2.26062	.405	-2.7273	9.9273
SOD level	DC	DPO	-7.20000*	1.78298	.014	-12.8153	-1.5847
		DMPO	-11.60000*	1.85930	.001	-17.4998	-5.7002
		DM	-13.40000	3.87320	.054	-27.0518	.2518
	DPO	DC	7.20000*	1.78298	.014	1.5847	12.8153
		DMPO	-4.40000	2.17386	.243	-11.0548	2.2548
		DM	-6.20000	4.03365	.470	-19.8028	7.4028
	DMPO	DC	11.60000*	1.85930	.001	5.7002	17.4998
		DPO	4.40000	2.17386	.243	-2.2548	11.0548
		DM	-1.80000	4.06796	.969	-15.4127	11.8127

	DM	DC	13.40000	3.87320	.054	-.2518	27.0518
		DPO	6.20000	4.03365	.470	-7.4028	19.8028
		DMPO	1.80000	4.06796	.969	-11.8127	15.4127

Note: Multiple comparisons were performed using Tukey HSD for Glutathione and MDA, while Games-Howell was used for SOD.

MD; mean difference, LB; lower bound, UB; upper bound, DM: diabetic group received only metformin, DMPO: diabetic group received combination of metformin and the extract, DPO: diabetic group received the extract only, DC: Diabetic control, MDA: Malondialdehyde, SOD: superoxide dismutase.

Contributions: Hiwa O. Abdalla, involved in the study design, experimental works, data collection & analysis, writing the first draft of the manuscript. Ridha H. Hussein, involved in study conception & design, supervision, critical revision, and guidance. Both authors confirmed and approved the final version of the manuscript.

Conflict of interest: the authors declare no competing interests.

Ethics approval: ethical approval was obtained from the Ethical Research Committee of the College of Science, University of Sulaimani (Approval No. 6, dated 24/8/2025).

Availability of data and material: all the raw data related to this study can be requested directly from the corresponding author upon reasonable request.

Funding: not applicable.

Artificial Intelligence (AI) – assistance: we have used GPT-5.5 solely for language revision and refinement.

Acknowledgments: we would like to express our sincere thanks to the Department of Biology, College of Science, University of Sulaimani, for providing us with the animal house and specialized lab for

experimental work. In addition, this study is derived from the Master's thesis titled "Modulatory effects of *Portulaca oleracea* extract on oxidative stress and reproductive hormones in diabetic male rats" submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Physiology in the Biology Department, College of Science, University of Sulaimani, Kurdistan Region, Iraq.