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Hepatic restorative potential of *Portulaca oleracea* ethanolic extract and/or metformin in type II *diabetes mellitus* model induced by high-fat diet and STZ

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Abstract

Background The risk of liver injury is increased by persistent hyperglycemia. Diabetes-related liver damage involves both oxidative stress and an abnormal inflammatory response. Nevertheless, many plants possess notable pharmaceutical properties, such as *Portulaca oleracea* (purslane, PE), which stands out for its wide range of potential medicinal applications globally. Consequently, this research sought to investigate the possibilities of purslane ethanolic extract in protecting against liver damage, oxidative stress, and the inflammatory response associated with diabetes.

Methods Diabetes was induced in rats through a high-fat diet, followed by a single intraperitoneal injection of 35 mg/kg of streptozotocin (STZ). The diabetic rats were treated with PE at dose of (100 mg/kg), metformin (MT) at dose of (100 mg/kg) or combination therapy of (50 mg/kg) PE plus (50 mg/kg) MT for 4 weeks. Prooxidants (TBARS, XO, and NO) levels, antioxidant enzymes activity (SOD and GPx), inflammatory markers (TNF- α , IL-1 β , and IFN- γ), and hepatic architecture abnormalities were measured by using standardized protocols.

Results Rats treated with STZ exhibited notable elevations in tissue prooxidants (TBARS, XO, and NO), and inflammatory markers (TNF- α , IL-1 β , and IFN- γ), with a parallel reduction in the activity of tissue antioxidants (SOD and GPx). Severe histopathological include vacuolated hepatocytes, dilated congested central veins, and numerous apoptotic signs, in addition to histochemical alterations in the liver tissue, were noted. When diabetic animals were treated with MT, PE ethanolic extract, or a combination of MT plus PE, these deleterious effects were improved.

Conclusion All together the obtained data confirmed that PE displayed an ameliorating effect against the liver damage, inflammatory response, and oxidative damage linked to *diabetic mellitus*.

Keywords Lipid peroxidation, Oxidative stress, Antioxidants, Inflammation, Liver injury

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Introduction

Type 2 *diabetes mellitus* (T2DM) is a prevalent, long-term metabolic problem (Liang et al., 2018), 382 million individuals are thought to be impacted globally, and it is anticipated that by 2035, this number will rise to 592 million (Reed et al., 2021). Chronic hyperglycemia affects several target organs, including the liver (Younossi et al., 2019). The unhealthy eating habits, such as diets high in fructose and saturated fats, enhance the risk of developing metabolic diseases like T2DM. The dramatic rise in fast food consumption, rich in fat and fructose, has led to insulin resistance, leptin resistance, oxidative stress, and the release of proinflammatory mediators (tumor necrosis factor alpha (TNF- α), interleukin 1 beta (IL 1- β), and interferon-gamma (IFN γ)) which are a few examples (Ekta et al., 2020).

In this sense, the pathophysiology of T2DM is influenced by oxidative stress, which is fundamental to hepatic damage. The primary cause of oxidative stress in liver cells is nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase), also known as inducible nitric oxide (NO) synthase activity, which is carried out by activated Kupffer cells (Mallik et al., 2024). Nonetheless, the liver possesses complex and powerful antioxidant mechanisms, including enzymes linked to glutathione (GSH), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GP χ), and superoxide dismutase (SOD). This defense mechanism hydrolyzes hydrogen peroxides and scavenges free radicals (Chun-Sik Bae et al., 2023). Substantial evidence supports the close association between inflammation and oxidative stress, as they both interact and exacerbate pathological states in some disorders (Zuo et al., 2019).

In diabetes, proinflammatory cytokines such as IFN- γ , TNF- α , and IL-1 β work together to up-regulate inducible nitric oxide synthase, increasing NO production (Chun-Sik Bae et al., 2023). Additionally, excess TNF- α production quickens the evolution of inflammation and β -cell death in pancreatic islets, which causes peripheral tissues to become even more insulin-resistant. Thus, an understanding of diabetes complications, such as diabetic-induced liver damage, requires an understanding of the management and interrelation of oxidative stress and inflammation. Moreover, attempts have been made to cure liver damage caused by T2DM and other disorders using antioxidant and anti-inflammatory therapy (Man-cak et al., 2024).

Metformin (MT) is one of the orally administrated drugs that are used to control hyperglycemia. Its effect is to decrease liver glucose synthesis and enhance insulin sensitivity by reducing gluconeogenesis, increasing glucose absorption in skeletal muscle, encouraging adipose tissue fatty acid oxidation, and enhancing peripheral

insulin sensitivity. Furthermore, metformin suppresses appetite, which lowers calorie intake and reduces body weight and total body fat (Jalali et al., 2020). Nevertheless, several attempts have been made to use antidiabetic therapy derived from natural substances such as purified compounds and fractions from edible and herbal plants.

A member of the *Portulacaceae* family is *Portulaca oleracea* L. Known by most as purslane, it is extensively distributed throughout tropical and subtropical regions of the world (Zhou et al., 2015). Traditional medicine has utilized purslane to treat a wide range of ailments, such as severe liver and other organ inflammations, fever, bladder ulcers, gastrointestinal, spleen, renal, and skin disorders. (Hadi et al., 2019). Purslane was designated as a "Global Panacea" by the World Health Organization due to its widespread use as a medicinal plant (Massaro Malheiros Ferreira et al., 2023). The chemical composition of PE extracts varies according to the area of cultivation. Still, it primarily consists of flavonoids, alkaloids, terpenoids, organic acids, and other naturally occurring substances like sterols, fatty acids, polysaccharides, terpenoids, vitamins, proteins, and minerals. (Li et al., 2023). Purslane is also rich in antioxidants, including α -tocopherol, ascorbic acid, glutathione, and melatonin (Makangali et al., 2024). According to a study by Hassan et al. (2023) purslane, metformin, or their combination may help reduce hyperglycemic, oxidative, and inflammatory burdens and enhance autophagy functioning in diabetic cognitively impaired albino rats. Thus, the present study examined whether MT and/or PE could improve liver dysfunction in diabetic rats. To accomplish this, the anti-inflammatory and antioxidant effects of both therapies were assessed.

Material and methods

Chemicals and assay kits

STZ (Cat. No. 18883–66-4) was purchased from Sigma-Aldrich Co. USA. MT was purchased from Merck Serono, Germany. Rat enzyme-linked immunosorbent assay (ELISA) kits of Rat TNF- α (Cat. No. ER1393), Rat IL-1 β (Cat. No. ER1094), and Rat IFN- γ (Cat. No. ER0012-CM) were purchased from Fine test Co., China. High-grade additional chemicals and solvents of related biochemical reagents were acquired from Sigma-Aldrich Co., USA.

Preparation of Purslane Extract

Using the procedures outlined in our earlier study (Bahr et al., 2022), PE extract was made. Briefly, the stems and leaves of the plants were cleaned and allowed to air dry at room temperature. After being pulverized, the dried plant was extracted using a 1:4 hydroalcoholic extraction ratio (70% ethanol) and then dried using a lyophilizer

(Mod. LyoConstellation S30, SP Scientific, USA) and kept at -20°C .

Experimental design and animal treatment

Forty male albino rats (4 months old) weighing 125 ± 20 g were purchased from the animal house of the Medical Technology Center, Alexandria University, Egypt. The animals were housed in the animal facility at Pharmaceutical & Fermentation Industries Development Center (PFIDC), City of Scientific Research & Technological Applications (SRTA-City), Egypt, in well-ventilated clean polycarbonate cages, maintained at a temperature of 22 ± 2.0 , humidity of 45% to 46%, and 12-h day-night cycle for 10 days for acclimatization before starting the experiment. Water and feed (varied per group) were given ad libitum. The PFIDC, SRTA-City Animal Ethics Committee gave its approval to all experimental procedures (IACUC #79-2A-0121).

The rats were randomly divided equally into five groups ($n=8$ each) as follows:

1. **Group I** (control group): the rats in this group fed on a typical balanced, commercial, rat meal.
2. **Group II** (Diabetic control group): the rats were fed on a freshly prepared high-fat diet (58% fat, 17% carbohydrate, and 25% protein; vitamins and minerals) for four weeks (Srinivasan et al., 2005), and then the diabetes was induced by single intraperitoneal injection of 35 mg/kg of STZ dissolved in 100 mM sodium citrate buffer, pH 4.5 after all night long fasting (Rahman & Venkatraman, 2011). Fasting blood glucose was measured 72 h after STZ injection to confirm diabetes induction, diabetic animals have fasting blood glucose concentration over than 200 mg/dl otherwise non-induced rats were excluded from experiment (Hassan et al., 2023). The diabetic animals were selected for the further treatments.
3. **Group III** (Diabetic+PE): the rats were fed on a freshly prepared high-fat diet for the period of experiment. Diabetes was induced as mentioned in the previous group, by single intraperitoneal injection of 35 mg/kg of STZ, the diabetes induction was confirmed when the fasting blood glucose concentration exceeded 200 mg/dl, 72 h after STZ injection, followed by daily treated with a 100 mg/kg oral dose of *purslane* ethanolic extract for four weeks (Hassan et al., 2023).
4. **Group IV** (Diabetic+MT): as previously declared, diabetes was induced to high-fat diet feeding rats by single intraperitoneal injection of STZ at a dose of 35 mg/kg. The animals considered diabetic once the fasting blood glucose concentration exceeded 200 mg/dl, 72 h after STZ injection (Hassan et al.,

2023), then the diabetic animals daily oral administrated with metformin at 100 mg/kg for four weeks (Srinivasan et al., 2005).

5. **Group V** (Diabetic+PE+MT): The high-fat diet feeding rats were given a single intraperitoneal injection of 35 mg/kg STZ to induced diabetes and then the diabetes induction was confirmed when the fasting blood glucose concentration exceeded 200 mg/dl, 72 h after STZ injection (Hassan et al., 2023). They were then treated orally each day with 50 mg/kg of PE and 50 mg/kg of MT for four weeks.

Biochemical assays preparation

Following the experiment, rats received an intraperitoneal injection of 100 mg/kg sodium pentobarbital to produce unconsciousness. The liver tissue was immediately removed, washed with a cold solution of 0.9% NaCl, frozen in liquid nitrogen, and stored at -80°C . Before centrifugation at 10,000 rpm for 10 min at 4°C , liver tissue was homogenized in a lysis solution that contained a protease inhibitor (pH 7.4, 150 mM NaCl, 1% Triton X-100, and 10 mM Tris). The resulting supernatant was subjected to lipid peroxidation, oxidative stress indicators, antioxidant enzymes, and inflammatory cytokine tests.

Estimation of prooxidants

Lipid peroxidation was measured in tissue homogenates via the thiobarbituric acid reactive substances (TBARS) assay according to the method detailed by Zalkin and Tappel (1960). This method was based on the reaction with thiobarbituric acid as a reagent under acidic and high-temperature conditions. At 532 nm, the colored product's absorbance was measured.

Nitric Oxide (NO) was estimated using Montgomery and Dymock's (1961) methodology, which is predicated on the production of pink azo-dye by the interaction of nitrite with sulfanilamide in an acidic media. At 540 nm, the color's intensity was determined using spectrophotometry.

The xanthine oxidase (XO) activity was measured by detecting the enzymatic conversion of xanthine to uric acid at 660 nm; this method was stated by Litwack et al. (1953).

Estimation of antioxidant enzymes activity (SOD and GPx)

The activity of glutathione peroxidase (GPx) was measured using the procedure outlined by Paglia and Valentine et al. (1967), which relied on the enzyme's capacity to degrade organic hydroperoxides when reduced glutathione (GSH) was present. Glutathione reductase (GR) converts the resultant oxidized glutathione (GSSG) back

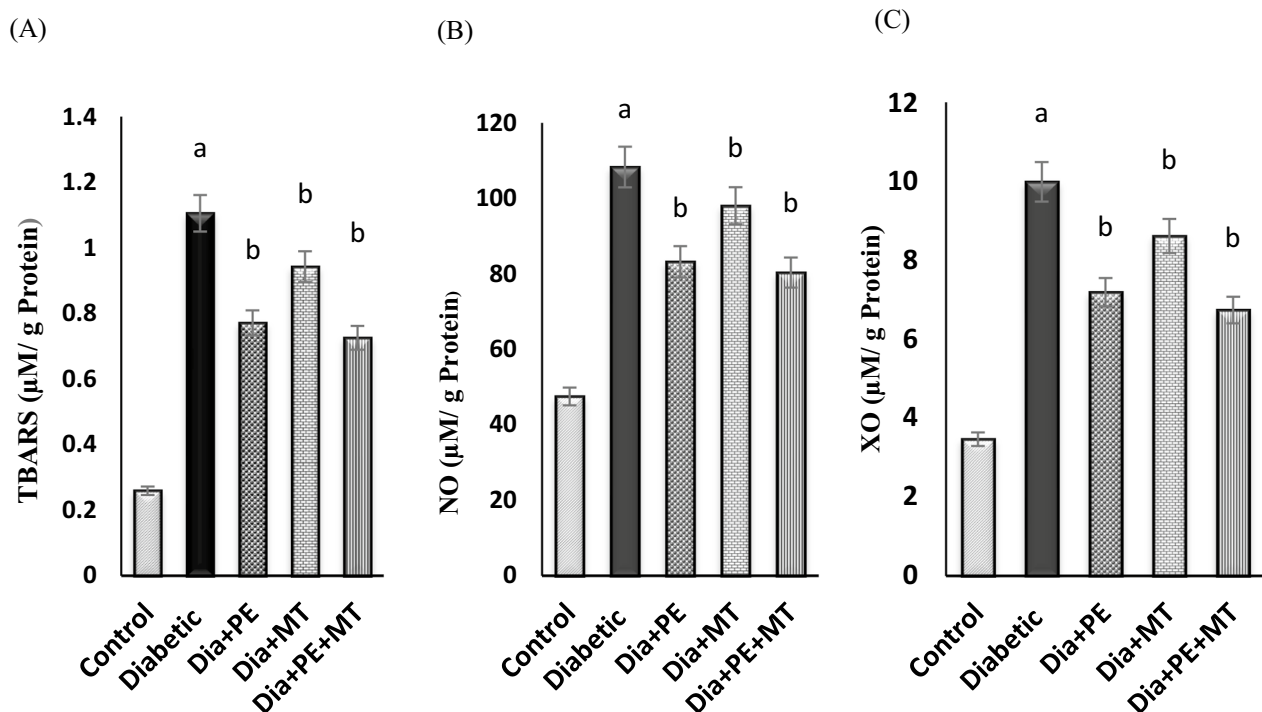


Fig. 1 Mean $\pm$ SD of **A** TBARS, **B** NO, levels, and **C** XO activity of the control and treated groups ($n=8$ each). **a** Highly significant to the control group $P<0.01$. **b** Highly significant to the diabetic group $P<0.01$

into GSH. GPx activity can be measured in the absorbance at 340 nm.

Superoxide dismutase (SOD) activity was investigated as Marklund and Marklund (1974) had reported. This technique is predicated on pyrogallol's suppression at alkaline pH. Autoxidation of pyrogallol results in the production of superoxide radicals (O_2^-). When SOD is present, the rate of pyrogallol autoxidation decreases, as determined spectrophotometrically at 420 nm.

Estimation of inflammatory cytokines (TNF- α , IL-1 β , and IFN- γ)

ELISA kits from Fine Test Co. in China were utilized to assess TNF- α , IL-1 β , and IFN- γ levels in the liver tissue. The sandwich enzyme-linked immune-sorbent assay technology was employed for detection, as directed by the manufacturer.

Histological examination

After removing the liver tissues, it was cleaned with saline, and fixed in a 10% neutral buffered formalin solution (pH 7.0), the tissues were dehydrated using ascending ethyl alcohol (50–99%) and then embedded in melted paraffin wax at 58–62°C. Tissue sections measuring 5 μ m in thickness were prepared and stained with Harris hematoxylin and eosin (H&E) (Harris, 1900) and Masson

trichrome stain to inspect the collagenous fibers (Bancroft & Stevens, 2008). The photographs were analyzed using a personal computer, camera software (Olympus DP74, Tokyo 163–0914, Japan), and high-power light microscopy (Olympus BX43F, Tokyo 163–0914, Japan).

Statistical analyses

The statistical significance differences of all measured data were ascertained by analyzing the mean differences using one-way analysis of variance (ANOVA). Tuckey's test was performed using Minitab 12 software (Product Code: 0471360619, Minitab LLC, State College, Pennsylvania, USA). Every data point is expressed using the mean $\pm$ SD of each group. The differences were considered statistically significant when $P<0.05$.

Results

Prooxidant and Antioxidant markers

The diabetic control group showed a highly significant ($p<0.01$) increase in the levels of TBARS (1.105 ± 0.097), NO (108.25 ± 1.23), and XO (9.98 ± 0.70) compared to the control group levels of TBARS (0.26 ± 0.027), NO (47.49 ± 1.33), and XO (3.5 ± 0.50) (Fig. 1A, 1B, and 1C, respectively) and a highly significant ($p<0.01$) decrease in the levels of SOD (55.00 ± 2.14) and GPx (6.67 ± 1.15), Comparable to the control group's SOD

(114.57 ± 1.74) and GPx (20.36 ± 0.55) levels (Fig. 2A and 2B, respectively).

On other hand, the administration of PE, MT, and the combination of both PE+MT induced a highly significant ($p < 0.01$) decrease in the levels of TBARS (0.77 ± 0.014 , 0.94 ± 0.045 , and 0.72 ± 0.024 , respectively), NO (83.20 ± 1.69 , 97.98 ± 1.56 , and 80.35 ± 1.65 , respectively), and XO (7.19 ± 0.61 , 8.61 ± 0.51 , and 6.74 ± 0.40 , respectively) than that of diabetic control group (Fig. 1A, 1B, and 1C, respectively).

Both diabetic+PE and diabetic+PE+MT groups showed the same statistically trend for SOD (Fig. 2A) and GPx (Fig. 2B) ($p < 0.01$), which were higher than those of the diabetic group. Moreover, the diabetic+MT group had SOD and GPx activities (63.17 ± 1.51 and 9.61 ± 0.58 , respectively) higher than that of the diabetic group, at $p < 0.05$.

Inflammatory markers

The levels of inflammatory cytokines (TNF- α , IL-1 β , and IFN- γ) (Fig. 3A, 3B, and 3C, respectively) exhibited a highly significant ($P < 0.01$) elevation in the liver of diabetic control rats (44.79 ± 1.60 , 230.93 ± 1.19 , and 47.77 ± 0.75 , respectively) compared to the control group (17.62 ± 0.76 , 95.96 ± 1.51 , and 24.47 ± 1.16 , respectively).

However, the treatment of diabetic groups with PE (33.12 ± 0.88 , 185.52 ± 1.51 , and 38.10 ± 0.35 , respectively), MT (37.03 ± 0.51 , 212.26 ± 1.27 , and $41.10 \pm$, respectively), and PE+MT (30.67 ± 0.77 , 179.50 ± 1.10 ,

and 35.41 ± 0.88 , respectively) showed a significant decrease in all evaluated liver inflammatory markers levels ($P < 0.01$) versus to those of diabetic control group.

Statistically, the current data reveal that the treatment with the combination of PE plus MT is the most effective against liver inflammation. While MT treatment has minimum anti-inflammatory influence.

Histological and histochemical studies

The liver of the diabetic control group revealed significant histological changes with H&E staining. Liver lobules were markedly disrupted with the absence of regular blood sinusoids, vacuolation, and notable dilated congested central veins. Hepatocytes exhibited varying signs, including nuclei that were fading, fragmented, or shrunken (Fig. 4C). Contrarily, the administration of PE, MT, or a combination of both revealed signs of improvement. These were evident in the regular blood sinusoids with normal hepatocyte configuration. The hepatocytes displayed all indicators of health, characterized by their polygonal shape, eosinophilic granulated cytoplasm, and centrally located spherical nuclei with distinct nuclear envelope, prominent nucleolus, and well-defined chromatin particles (Fig. 4E, G, I, respectively). Histochemical investigation using Masson's trichrome staining showed increased collagen fiber deposition in the vicinity of the central vein in the diabetic control rats when compared to the control rats (Fig. 4D). On the other hand, administration of PE and/or MT reduced the increase in collagen

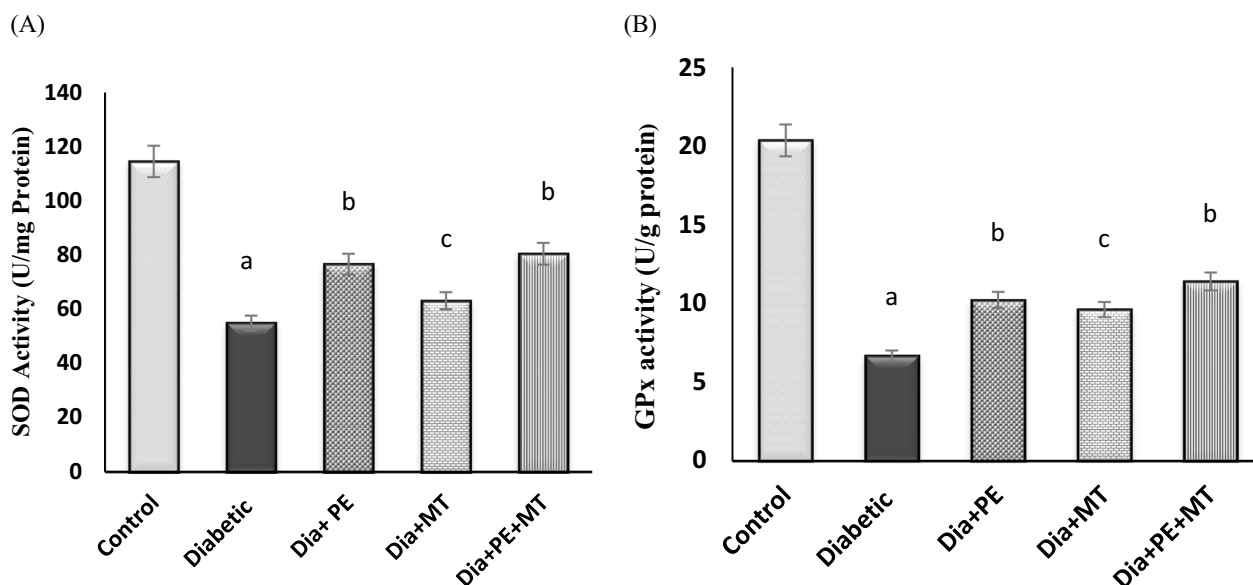


Fig. 2 Mean $\pm$ SD of **A** SOD and **B** GPx levels of the control and treated groups ($n = 8$ each). **a** Highly significant to the control group $P < 0.01$. **b** Highly significant to the diabetic group $P < 0.05$. **c** Significant to the diabetic group $P < 0.01$

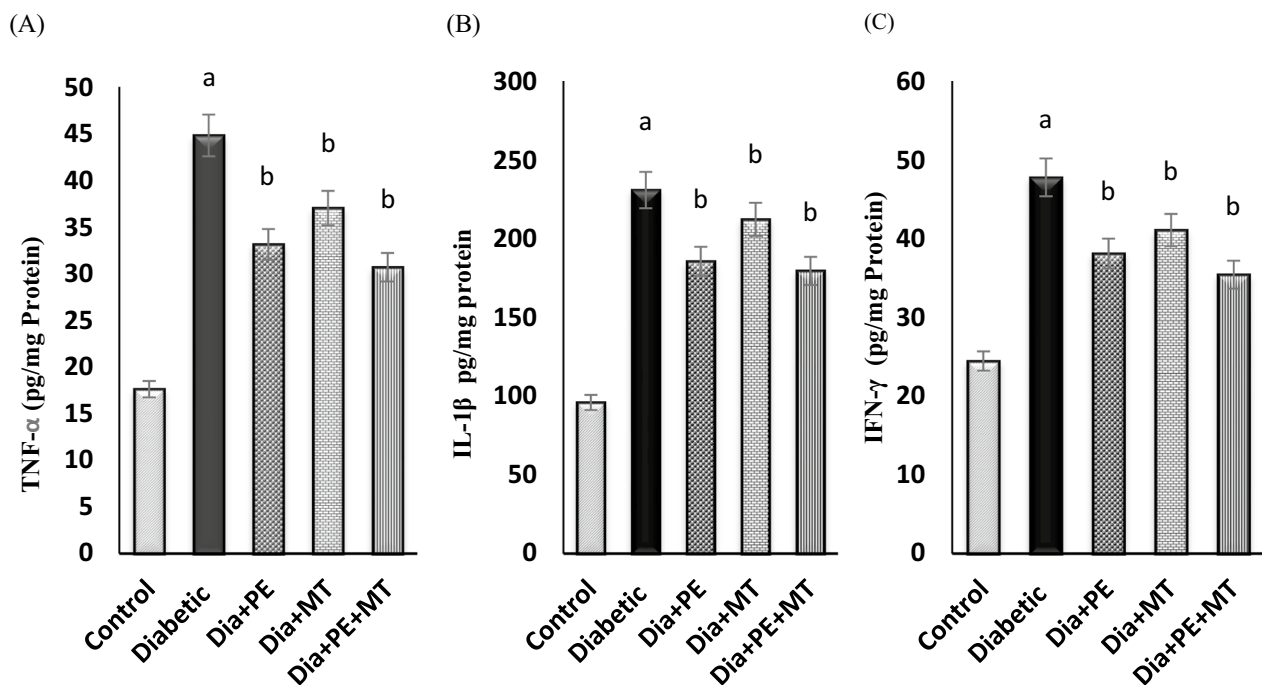


Fig. 3 Mean $\pm$ SD of **A** TNF- α , **B** IL-1 β , and **C** IFN- γ levels of the control and treated groups (n=8 each). **a** Highly significant to the control group $P < 0.01$. **b** Highly significant to the diabetic group $P < 0.01$

fiber deposition around the central vein (Fig. 4F, H, J, respectively).

Discussion

Since the liver is essential for controlling blood sugar levels, type 1 and type 2 diabetes can both affect liver function in distinct ways. Diabetic individuals have a high frequency of liver disorders (Zhu et al., 2020). In the same line, our previous study (Bahr et al., 2022) demonstrated that the HFD-received and STZ-injected diabetic rats' exhibited increased glucose levels; furthermore, the liver enzyme activity was assessed and reported to prove diabetic-induced liver dysfunction, whereas elevated ROS generation and an aberrant inflammatory response have been connected to hyperglycemia-mediated liver damage (Al-Amarat et al., 2021). The current study demonstrates a tight relationship between T2DM and the liver's oxidative stress and inflammatory response. Here, the diabetic

control liver displayed excess lipid peroxidation and reactive oxygen species (ROS) levels as indicated by elevated TBARS, NO levels, and XO activity, and suppressed antioxidant enzymes activity such as SOD and GPx. Supporting studies by Unsal et al. (2020) and Sarkar et al. (2018) showed that the liver of streptozotocin-induced T2DM rats contained elevated amounts of TBARS, a lipoperoxidation byproduct, and increased XO activity. This explanation outlines the relationship between T2DM, TBARS, NO, and XO, and these findings align with our findings.

The enzyme XO plays a significant role in generating ROS, which are key contributors to oxidative stress. The generation of ROS by XO occurs during the oxidation of hypoxanthine and xanthine to uric acid. This process is part of the natural metabolic pathway. However, too much XO activity might activate the body's defenses against oxidative damage (Zhang et al., 2019). Furthermore, the production of NO, which harms cells and is

(See figure on next page.)

Fig. 4 **a, b** Photomicrographs of the liver section from the control group stained with H&E and Masson trichrome stains, respectively. **c, d** Photomicrographs of the liver section from the diabetic group stained with H&E and Masson trichrome stains, respectively. **e, f** Photomicrographs of the liver section from the diabetic + PE group stained with H&E and Masson trichrome stains, respectively. **g, h** Photomicrographs of the liver section from the diabetic + MT group stained with H&E and Masson trichrome stains, respectively. **i, j** Photomicrographs of the liver section from the diabetic + PE + MT group stained with H&E and Masson trichrome stains, respectively. Central vein (CV), endothelium (arrow), hepatocyte (H), Kupffer cell ($\blacktriangleright$), blood sinusoid ($\sim\sim\sim$), congested vein (Cong), pyknotic nucleus ($\gg\gg$), hyperchromatic nuclei ($\blacktriangleleft$), nuclear fading ($\rightarrow\blacktriangleright$), nuclear fragmentation ($\blacktriangleright\rightarrow$), karyomegaly ($\blacktriangleright$), Masson trichrome positive reaction (thick arrow). (Magnification, $\times 400$)

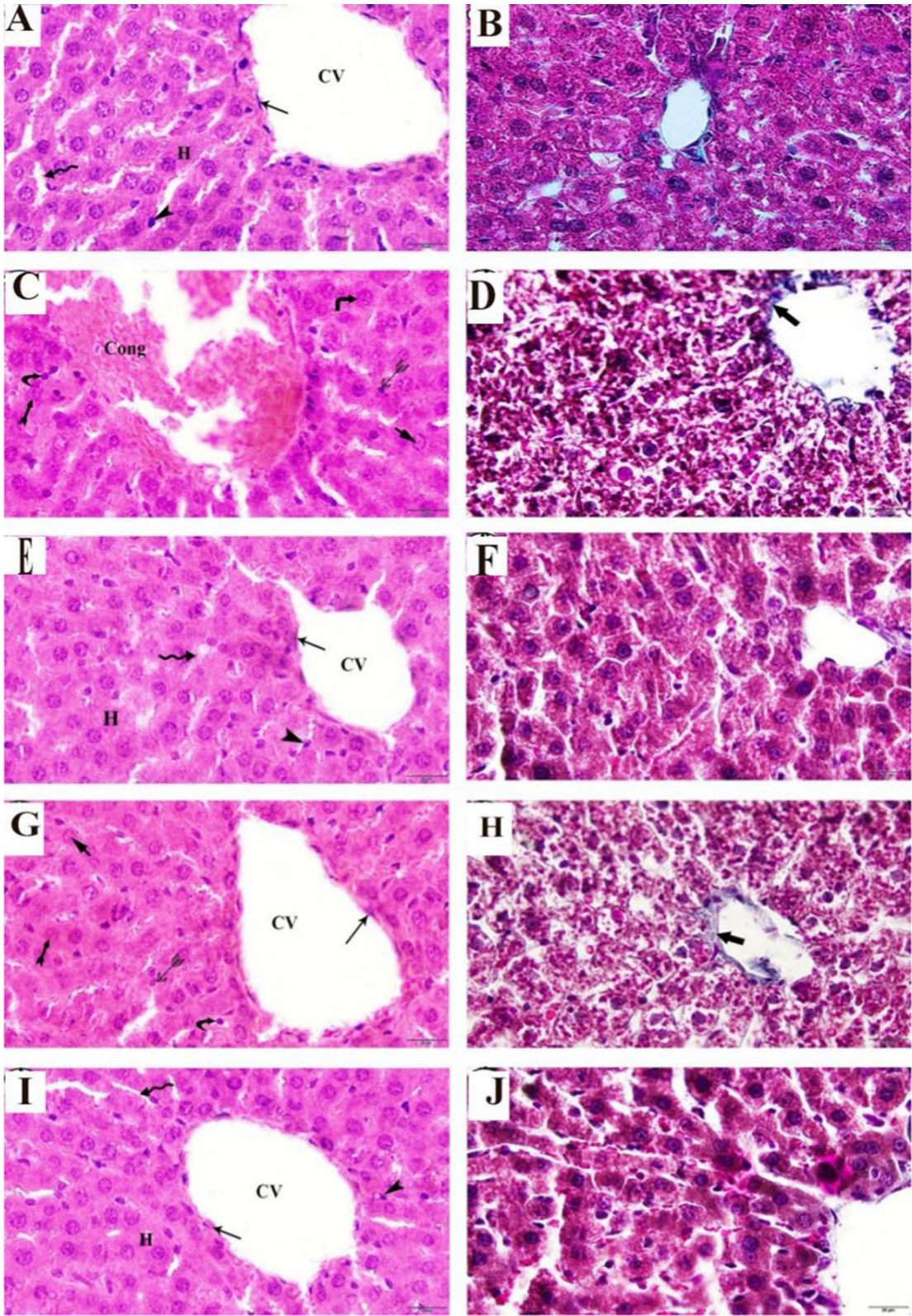


Fig. 4 (See legend on previous page.)

eventually to blame for the late complications of both types of diabetes, is a significant outcome of the activation of stress-sensitive signaling pathways (Hu et al., 2020).

Regarding antioxidant activity, the current study exhibits SOD and GPx activity suppression; these results are in line with numerous studies that demonstrated alterations in antioxidant defenses, encompassing both the non-enzymatic and enzymatic antioxidant systems, as well as the involvement of free radicals in the development of diabetes (Mirza et al., 2022). In this line, reduced levels of SOD, catalase, and GPx were seen in certain investigations assessing their activities in individuals with T2DM (Behrouj et al., 2018). Further supporting evidence was supplied by Vega Joubert et al. (2022), who found the function of enzymatic and non-enzymatic antioxidants in the prevention/treatment of T2DM and its related problems.

Concerning the inflammatory response, diabetes and inflammation are closely connected, and inflammation plays a significant role in the development and progression of T2DM. Chronic inflammation is a key factor contributing to insulin resistance, β -cell dysfunction, and diabetic complications (Khalid et al., 2021). Likewise, a study published by Chen et al. (2020) revealed that ROS produces proinflammatory/inflammatory mediators that lead to liver damage. The T2DM-induced rats in the current study exhibited increased TNF- α , IL-1 β , and IFN- γ levels; these findings are in harmony with Bowker et al. (2020); Khalid et al. (2021); Lv et al. (2021). Inflammation can interfere with the normal action of insulin, leading to higher blood glucose levels.

Interestingly, the current findings indicated that groups treated with PE ethanolic extract MT, or a combination, significantly reduce oxidative stress and enhance liver antioxidant levels. These results align with a study by Desta et al. (2020); Jafari et al. (2023), which demonstrated that purslane has antioxidant properties, as evidenced by the correlation between increased glutathione levels and reduced malondialdehyde (MDA) and NO in rats, along with elevated levels of GPx, glutathione reductase (GR), glutathione-s-transferase (GST), catalase (CAT), and SOD. Similarly, Kumar et al. (2022); Aladai-leh et al. (2019) suggested that using PE ethanolic extract might offer protection against STZ-induced damage, possibly by reducing oxygen free radicals.

Remarkably, our data revealed that proinflammatory markers TNF- α , IFN- γ , and IL-1 β were significantly reduced when treating T2DM rats with PE or a combination of PE and MT. These findings are in line with reported data by Nkobile et al. (2023) where pretreatment with an aqueous extract of PE notably decreased TNF- α and IL-6 levels in diabetic rats. Similarly, Jalali

et al. (2022) demonstrated that a polysaccharide extract from purslane significantly reduced IL-6 and TNF- α levels in diabetic rats. Similarly, Baradaran Rahimi et al. (2024) indicated that several alkaloids derived from PE possess anti-inflammatory effects. Additionally, PE treatment may improve insulin signaling and glucose uptake by reducing proinflammatory molecules. Moreover, Nkobile et al. (2023); Jalali et al. (2022); and Baradaran Rahimi et al. (2024) suggested that PE may help reduce IFN- γ levels, thereby mitigating inflammation and oxidative stress in diabetic models; PE may help regulate immune function by decreasing the overactivation of the immune system that leads to the production of IFN- γ and other inflammatory cytokines. It might work by inhibiting the signaling pathways that produce these cytokines, such as nuclear factor kappa (NF- κ B) and other proinflammatory transcription factors. Furthermore, alterations in liver function markers were found to be associated with histopathological changes.

This study observed significant liver damage in the induction group, including disruption of liver lobules, loss of hepatic cords, and varying degrees of vacuolation in hepatocytes with pyknotic nuclei. There was also congestion, dilation of central veins, and necrotic changes in some hepatocytes, such as nuclear fading, fragmentation, and shrinkage. These findings align with reported data by Chen et al. (2018), who reported similar liver changes in diabetic mouse models induced by a high-fat diet and STZ, including hepatocellular necrosis, inflammatory cell infiltration, and fatty degeneration around the central veins. Additionally, Wickramasinghe et al. (2024); and Dwivedi and Jena (2020) observed hydropic degeneration and lobular inflammation in the liver tissue of HFD-fed STZ-induced rats. The liver damage observed in this study is consistent with the explanations provided by De La Rosa et al. (2022), who linked diabetes-induced liver injury to insulin resistance, hyperglycemia, and advanced glycation end products. Chronic hyperglycemia increases reactive oxygen species (ROS), leading to oxidative stress that damages liver cells.

The histological observation of DM rats' liver sections revealed increased collagen fiber deposition in the areas surrounding the central vein boundary. These findings could be reconciled with Kong et al. (2019); and Jiang et al. (2018), which clarified that the generation of hydroxyl reactive oxidizing molecules by the biological system was responsible for the increased collagen creation. This, in turn, caused lipid peroxidation, which in turn damaged proteins and nucleic acids. Another study reported by Tanwar et al. (2020) revealed that because ROS promotes the growth of hepatic stellate cells, they raise the synthesis of collagen, which aggravates and accelerates hepatic fibrosis.

Conversely, the groups administered with purslane ethanolic extract and/or metformin revealed notable signs of improvement, as indicated by the normal arrangement of hepatic cords and blood sinusoids around the central vein, with the hepatic lobules retaining their typical histological structure. The hepatocytes appeared healthy, showing a polygonal shape, eosinophilic, granulated cytoplasm, and one or occasionally two centrally located spherical nuclei. The nuclei had a prominent nucleolus and visible chromatin particles. In addition, a nearly normal distribution of collagen fibers is noticed. Our results are in line with those of Mousa et al. (2021); de Souza et al. (2022); and Binici et al. (2021), who reported significant recovery with purslane treatment, including reduced liver fibrosis, normalized hepatic lobules, healthy hepatic parenchyma, and intact blood sinusoids. Purslane's hepatoprotective effects are ascribed to its anti-inflammatory, regenerative, and antioxidant qualities, which may help prevent or repair liver damage. Nonetheless, these impacts are consistent with what we have seen.

Conclusion

Our current findings demonstrated that concurrent PE extract administration reduced the adverse effects of type 2 diabetes due to its anti-inflammatory and antioxidant qualities. Consequently, the PE extract's anti-inflammatory, antidiabetic, and antioxidant properties show promise as a therapeutic measure against both forms of diabetes mellitus. These results also point to intriguing avenues for additional clinical research into the potential benefits of PE extract, either by itself or in combination with MT, in bolstering the course of treatment for type II diabetes mellitus.

Abbreviation

CAT	Catalase
DM	<i>Diabetes mellitus</i>
GP _x	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
HFD	High fat diet
IFN γ	Interferon gamma
IL 1- β	Interleukin 1 beta
IL-6	Interleukin 6
MDA	Malondialdehyde
MT	Metformin
NADPH	Nicotinamide adenine dinucleotide phosphate
NF- κ B	Nuclear factor kappa
NO	Nitric oxide
O ₂ ⁻	Superoxide radicals
PE	Purslane
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STZ	Streptozotocin
T2DM	Type 2 diabetes mellitus
TBARS	Thiobarbituric acid reactive substances

TNF- α	Tumor necrosis factor alpha
XO	Xanthine Oxidase

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Author contributions

Abd El-Kader M. Abd El-Kader: Supervise the conducted research, contributed to the research methodology, data analysis, and paper writing revision; Maha A. El Demellawy: Supervision, validation, provided essential reagents and materials and participated in paper writing; Doaa A. Ghareeb: designed research, methodology, data analysis, docking revision, provided essential reagents, materials, and resources. Amna H M Nour: Carried out and interpreted the histological examinations, and paper writing; Naglaa bahr: Conducted research, data analysis, performed the statistical analysis, and paper writing. All authors have read and approved the final manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval consent to participate

The animal care conformed to the guide for the care and use of laboratory animals according to the Standing Committee's Guide for Scientific research ethics-SRTA-City, with reference number, IACUC #79-2A-0121. In addition the manuscript adheres to ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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