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Experimental Research Article

Endurance exercise and Urtica Dioica extract ameliorate TLR-4 levels and myocardial inflammatory profile in STZ-induced diabetic rats

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ABSTRACT

Introduction: In diabetic patients, high blood sugar levels provoke sustained inflammation in cardiac tissue and impair normal cardiac activity. Considering the anti-diabetic characteristics of endurance training and Urtica Dioica (UD) extract, this study evaluated their potential in modulating TLR-4 levels and the inflammatory condition of cardiac tissue in diabetic Wistar rats.

Methods: In the present study, a total of 50 male Wistar rats were randomly allocated into 5 equal groups: Healthy-Control (H-C), Diabetic-Control (D-C), Diabetic-Exercise (D-Ex), Diabetic-Urtica Dioica (D-UD), and Diabetic-Exercise-Urtica Dioica (D-Ex-UD). Diabetes was induced by an intraperitoneal injection of streptozotocin (STZ) (45 mg/kg). Two weeks post-STZ injection, a moderate-intensity treadmill running regimen (five days/week) was performed, and UD extract (50 mg/kg) was given per day by gavage for 6 weeks.

Results: A six-week regimen of exercise and UD extract improves diabetes-induced weight loss. They also resulted in a significant decrease in blood glucose of the D-Ex ($P = 0.001$), D-UD ($P = 0.001$), and D-Ex-UD ($P = 0.001$) groups relative to D-C. Moreover, these interventions alone and in interaction resulted in a decrease in TLR-4 and TNF-α levels, as well as an increase in IL-10 levels in cardiac tissue. However, the D-Ex-UD group indicated the most excellent effectiveness in reducing TLR-4 levels and improving the cardiac inflammatory profile (IL-10/TNF-α ratio).

Conclusion: Endurance exercise and UD extract reduce myocardial inflammation by modulating the TLR-4 pathway and restoring the balance of inflammatory cytokines, such that the combined intervention, through simultaneous targeting of inflammatory pathways, exerts a stronger effect than either intervention alone.

Keywords:
Endurance exercise
Urtica Dioica
Inflammation
Cardiac tissue
Diabetes

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Introduction

Diabetes mellitus (DM) is a prevalent metabolic disorder globally and poses a significant risk factor for cardiovascular complications. According to a 2019 study, the worldwide prevalence of diabetes was estimated at 463 million people (9.3%), a figure expected to rise to 578 million (10.2%) by 2030 (Saeedi et al., 2019). Diabetes is a chronic metabolic disorder characterized by persistent hyperglycemia, which occurs in both type 1 and type 2 diabetes. In type 1 diabetes (T1D), hyperglycemia primarily results from the autoimmune destruction of pancreatic beta cells in the islets of Langerhans, leading to an absolute deficiency in insulin secretion. In contrast, type 2 diabetes (T2D) is characterized by a combination of insulin resistance in peripheral tissues and a relative deficiency in insulin secretion (Samadian et al., 2021). Irrespective of its type, diabetes-associated hyperglycemia broadly impairs the structure and physiological function of multiple organs. One major complication is diabetic cardiomyopathy (DCM), a frequently observed cardiovascular condition among diabetic patients. This condition significantly affects heart function in individuals with type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM). DCM is associated with molecular, structural, and functional changes in the myocardium that can ultimately lead to heart failure. Although the precise mechanisms underlying diabetic cardiomyopathy (DCM) are not fully understood, several interrelated factors have been identified as key contributors. Metabolic abnormalities, inflammatory responses, increased oxidative stress, and fibrosis serve as key mediators in the onset and progression of DCM (Huang et al., 2023).

Recent evidence indicates that activation of Toll-like receptors (TLRs), particularly Toll-like receptor 4 (TLR-4), plays a central role in myocardial inflammation and the development of diabetic cardiomyopathy (DCM). TLR-4 is the most abundantly expressed TLR in cardiac tissue and is activated by pathogen- and damage-associated molecular patterns (PAMPs and DAMPs), which are elevated under diabetic conditions (Kostrzewa-Nowak et al., 2020). Upon activation, TLR-4 initiates intracellular signaling primarily through the Myeloid Differentiation Factor 88 (MyD88)-dependent pathway, resulting in the activation of stress-related kinases such as JNK, IKK, and p38, and ultimately the transcription factor NF-κB. NF-κB activation promotes the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, which contribute to myocardial inflammation, cardiac hypertrophy, fibrosis, and cardiomyocyte apoptosis (Fuentes-Antrás et al., 2014). In this context, inflammatory biomarkers such as TNF-α and IL-10 are commonly used to assess inflammatory status, disease progression, and therapeutic efficacy in diabetic cardiovascular complications (Barry et al., 2016; Parente et al., 2024). TNF-α exacerbates insulin resistance by impairing insulin signaling and GLUT4-mediated glucose uptake, and its levels are typically elevated in diabetes (An et al., 2015; Yaturu et al., 2008). In contrast, IL-10 exerts anti-inflammatory effects by suppressing pro-inflammatory cytokine production, improving insulin sensitivity, and supporting pancreatic β-cell function. Consequently, the IL-10/TNF-α ratio is considered a reliable indicator of inflammatory balance in diabetic conditions (dos Santos Haber et al., 2023).

Conventional pharmacological treatments for the prevention and management of DCM have limited efficacy and can be costly, thus creating a need for novel therapeutic strategies. Recently, research has focused on combination approaches, including herbal medicines and exercise interventions, to target diabetes-induced myocardial inflammation (Rahmati et al., 2021; Rajabi et al., 2024). Exercise, as a non-pharmacological intervention, extends beyond blood glucose control to protect the heart against DCM and improve its function by influencing key pathological pathways (apoptosis, fibrosis, and hypertrophy). Regular physical activity contributes to improved heart function by reducing ROS and pro-inflammatory factors (TNF-α, IL-1β) and increasing anti-inflammatory factors (IL-10) (Ghaffari et al., 2023; Rajabi et al., 2024). Moreover, exercise has been shown to reduce apoptosis and inflammation through modulation of TLR-4 signaling (Wu et al., 2014). However, it should be noted that the cardiovascular effects of exercise depend on its type, intensity, and duration. Medicinal plants, particularly as extracts, have gained attention as complementary therapeutic strategies for diabetes (Ademolu 2019). Among them, Urtica dioica (Urticaceae), widely distributed in South Asia, exhibits notable hypoglycemic and anti-inflammatory properties (Morshed et al., 2011). Experimental evidence indicates that Urtica dioica leaf extracts can stimulate insulin secretion and exert insulin-mimetic effects in both healthy and streptozotocin-induced diabetic models (Chehri et

al., 2022). Recent studies have further confirmed the anti-inflammatory and glucose-lowering effects of Urtica dioica in diabetic conditions (Keshvari et al., 2020; Rahmati et al., 2021). These effects are attributed to its bioactive phytochemicals, including flavonoids, phenolic compounds, and phytosterols (Morshed et al., 2011; Parente et al., 2024). Among these, quercetin and scopoletin have been shown to modulate inflammatory mediators and key signaling pathways such as TLR-4 and NF-κB, leading to reduced pro-inflammatory cytokine production (Byun et al., 2013; Choi RaYeong et al., 2017; Zhao et al., 2021). Collectively, these findings highlight the therapeutic potential of Urtica dioica in the management of diabetes-related inflammatory complications. The novelty of the present study lies in the simultaneous investigation of the combined effects of endurance exercise and Urtica dioica extract on the TLR-4 pathway and myocardial inflammatory profile in an STZ-induced diabetic rat model, as most previous studies have examined these interventions separately. We hypothesized that the combined intervention would more effectively reduce cardiac inflammation through modulation of TLR-4 compared with either treatment alone. Therefore, the primary aim of this study was to evaluate the individual and combined effects of endurance exercise and nettle extract on myocardial TLR-4 levels and inflammatory markers in STZ-induced diabetic rats.

Material and Methods

Experimental animals

In this experimental study, we used an experimental design with a post-test control group. For this purpose, fifty male Wistar rats (6 weeks old, mean weight 232.74±12.42 g) were acquired from Lorestan University of Medical Sciences for this experiment. Following their transfer to the laboratory, the animals were acclimatized for one week under standard conditions, including a 12-hour light/dark cycle, controlled temperature (25 ± 1°C), and unrestricted access to water and standard pellet diet. Two weeks into the study, the rats were familiarized to treadmill-based endurance training (10-15 min, 5-10 m/min, and 5 d/w). Acoustic cues were applied to stimulate movement, and the treadmill's front area was lined with thick paper in order to direct the animals toward the front. The rats were split into five equal groups at random, which included Diabetic-Control (D-C), Healthy-Control (H-C), Diabetic-Exercise (D-Ex), Diabetic-Urtica Dioica (D-UD), and Diabetic-Exercise-Urtica Dioica (D-Ex-UD). All animal handling procedures in this research were carried out in compliance with the guidelines of the National Institutes of Health (NIH) and were approved by the Research Ethics Committee of Lorestan University (IR: LU.ECRA.2019.17). Figure 1 presents a schematic representation of the study protocol conducted over a 10-week period. It is worth noting that, to avoid confusion, time points in the result figures (W2, W4, W6) refer to weeks after the initiation of interventions, not to the total study duration shown in the experimental timeline.

Induction of diabetes

For two weeks prior to the experiment, all rats were kept in the animal laboratory to reach the optimal weight (at least 220 g). After an overnight fast, to establish a model of experimental diabetes, we administered a single intraperitoneal injection of streptozotocin (45 mg/kg; Sigma, St. Louis, MO) dissolved in 0.5 mol/L citrate buffer (pH 4.0), whereas control rats were administered the same volume a solution of citrate buffer (Rahmati et al., 2015). The rats showed no symptoms of behavioral abnormalities or physiological disorders such as inflammation or digestive issues following the STZ injection. To confirm the induction of diabetes, blood samples were taken 48 hours following the injection by creating a tiny incision on the tail vein with a lancet, and they were examined with a glucometer (Emperor, Korea Isotech). Diabetic rats were defined as having fasting blood glucose levels of 300 mg/dL or above. Following the STZ injection, for two weeks, the rats were maintained in the laboratory environment for adaptation without any intervention (Bostani et al., 2020). Every two weeks, the blood glucose (BG) and body weight (BW) of every rat were measured.

Plant gathering, extract preparation, and dosage

The process began with the collection of leaves of UD from the mountain's region of Lorestan Province. The leaves were first verified by an expert at the Lorestan Farming Authority, who recorded the sample as specimen no. 13776. Next, the leaves were dried away from direct sunlight and pulverized. To create the hydroalcoholic extract, 500 g of this powder was steeped for 18 hours in a solution of 70% ethanol and 30% water. The

final extract was then twice filtered through Whatman filter paper to remove impurities (Keshvari et al., 2020). In the next step, in order to concentrate the solvent and remove the ethanol, the extract of UD was placed at 60 °C. Then, to remove all ethanol and prepare the dry extract, the hydro-alcoholic solution was incubated at 60°C for 24 hours (Keshvari et al., 2020). To identify and quantify the bioactive constituents of the Urtica dioica extract, gas chromatography-mass spectrometry (GC/MS) was employed. For this procedure, 0.3 g of the dry extract was reconstituted in 1 mL of water and centrifuged for 3 minutes at 16,000 rpm and a temperature of 4 °C. Subsequently, a 2 µL sample of the solution was introduced in split mode for GC/MS analysis, which was conducted using the Agilent 7890A gas chromatograph connected to a 7000C triple quadrupole mass analyzer (Keshvari et al., 2020). Two weeks after diabetes induction, administration of Urtica dioica extract was initiated. The dried plant extract was administered at a dose of 50 mg/kg of body weight, dissolved in distilled water, and delivered by oral gavage once daily for six weeks to the D-UD and D-Ex-UD groups. The gavage volume for each animal was calculated and adjusted according to its body weight. To control for the potential effects of gavage volume, animals in the groups not receiving the extract were administered the same gavage volume (expressed as mL/kg of body weight) consisting solely of distilled water (Keshvari et al., 2020; Patel et al., 2016).

Treadmill exercise

The endurance training protocol consisted of 6 weeks, five sessions per week, performed on an animal treadmill (Azarakhsh, Pishro Andisheh Company, Tehran, Iran) at moderate intensity. A training session comprised three phases: a 3-minute warm-up, a main exercise period lasting 10–30 minutes, and a 3-minute cool-down.. The study period progressively increased exercise intensity and duration. In the initial week, the training protocol began with a 10-minute session at 10 m/min and gradually progressed to 18 m/min for 30 minutes by the sixth week. The training intensity was gradually increased over a four-week period. The rats ran at a speed of 10 m/min for 20 minutes during the second week, followed by 15 m/min for 20 minutes in week 3. In week 4, the duration was extended to 30 minutes at the same speed, and in week 5, the speed was increased to 18 m/min for 30 minutes. To ensure stabilization of the physiological adaptations, all training parameters were held steady during the final week of the protocol. Furthermore, throughout the exercise protocol, animals in the training groups (D-Ex and D-Ex-UD) did not exhibit any signs of severe fatigue or motor impairments (Rahmati et al., 2015).

Tissue preparation

Two days following the final endurance training session and administration of the UD hydro-alcoholic extract, all experimental rats were subjected to chloroform anesthesia. Perfusion was performed using 100 mL of saline solution to clear the blood, followed by a 4% paraformaldehyde solution, in a volume of 250 mL prepared in 0.1 M a phosphate-based buffer at a pH of 7.4. After anesthesia, the chest area was split, and the heart was carefully separated from the animal's body under sterile conditions immediately after separation and washing with saline solution. It was stored in a freezer at -80 °C for further analysis.

Western blot analysis

TLR-4 protein levels were measured using Western blotting according to previous research (Rahmati and Kazemi 2019). We used polyclonal antibodies for TLR-4 (TLR4 (25): 1:1000; sc-293072) and for β-actin (β-actin (C4): 1:1000; sc-47778). For data analysis and normalization, the relative levels of each protein on the same membrane were quantified relative to β-actin, which functioned as the internal reference protein.

Biochemical assays for TNF-α and IL-10

To conduct biochemical tests, 100 mg of tissue was ground and homogenized with a mortar and pestle. The homogenized tissue was then suspended in phosphate-buffered saline (PBS) containing protease inhibitor (PI) and phenylmethylsulfonyl fluoride (PMSF) and sonicated using an ultrasonic device in three 40-second cycles (with 10-second intervals). After centrifugation (20 min at 4000–6000 rpm), the supernatant was collected for further analysis. TNF-α and IL-10 levels were measured using Cusabio rat-specific ELISA kits. The TNF-α kit had a sensitivity of 1.56 pg/ml and a range of 6.25–400 pg/ml (intra-assay CV: 2.7–5.2%; inter-assay CV: 4.9–9.5%). The IL-10 kit showed a sensitivity of 0.078 pg/mL and a range of 3.12 to 200 pg/mL (intra-assay CV: 0.2 to 4.2%; inter-assay CV: 3.3 to 6.4%).

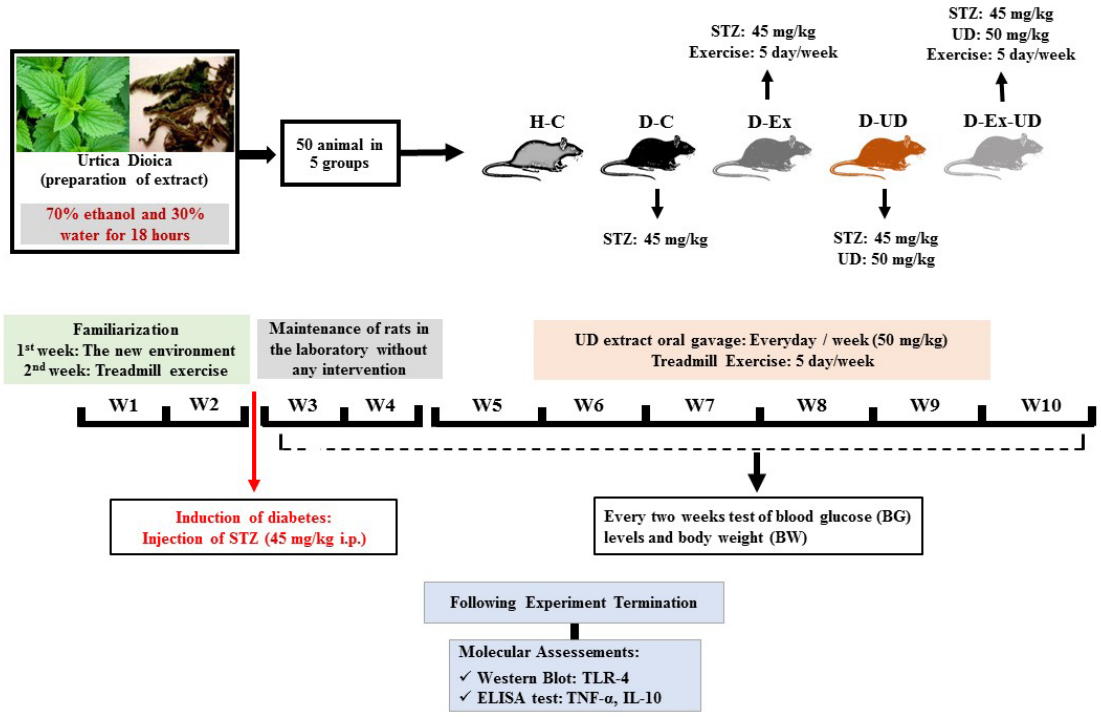

FIGURE 1. Summary of the experimental design for 10 weeks: Before starting the study, the steps of preparing the Urtica Dioica (UD) extract were performed. Then the animals were familiar with the laboratory environment and treadmill, and in the next step, diabetes was induced by injecting STZ (45 mg/kg, IP) in the diabetic groups. After these measures, all the animals were kept in the laboratory for two weeks without any intervention, and then the treatment with Ex and UD started for 6 weeks. Finally, animals were sacrificed 48 hours after the last intervention for molecular studies. D-C: Diabetes-Control, H-C: Health-Control, D-Ex: Diabetes-Exercise, D-UD: Diabetes- Urtica ioica, D-Ex-UD: Diabetes-Exercise- Urtica Dioica.

Statistical analysis

Data analysis was performed using SPSS version 20. Data normality and variance homogeneity were tested using the Shapiro–Wilk and Levene's tests, respectively. Repeated-measures ANOVA was employed to evaluate changes in glucose and rat body weight throughout the exercise period. Additionally, one-way ANOVA was used for statistical analysis of research variables, and, following ANOVA, Tukey's post hoc test was employed to identify specific group differences. Furthermore, ImageJ software calculated the intensity of the Western blot bands, and GraphPad Prism software was used to generate graphs. The results are presented as Mean ± SD, and a P-value of less than 0.05 was considered statistically significant.

Results

The Effects of Ex and UD Interventions on Body weight (BW) in Diabetic Rats

Changes in BW of rats in different experimental groups during the implementation of the research protocol are illustrated in Fig. 2. At baseline (pre-STZ), no significant differences in BW (232.74±12.42 g) were observed among the groups (one-way ANOVA, $F(4,45)=0.284$, $P=0.511$). Mauchly's test indicated that the assumption of sphericity was violated for the time factor ($W=0.311$, $\chi^2(9)=50.66$, $P<0.001$); therefore, Greenhouse–Geisser correction was applied. Repeated measures ANOVA demonstrated a significant effect of time on BW ($F(2.709, 121.88)=11.210$, $P=0.001$), a significant effect of group ($F(4,45)=34.548$, $P=0.001$), and a significant time×group interaction ($F(10.835, 121.88)=26.518$, $P=0.001$), indicating different BW change patterns among the groups over time. Post hoc comparisons indicated that two weeks (W2) post-STZ injection, BW in the diabetic groups significantly decreased compared to the H-C group ($F=0.185$, $P=0.001$), and this decrease continued until the end of the sixth week ($F=19.23$, $P=0.001$). The mean BW values of the D-C group were significantly lower compared with the D-Ex, D-UD, and D-Ex-UD groups during the six-week period after

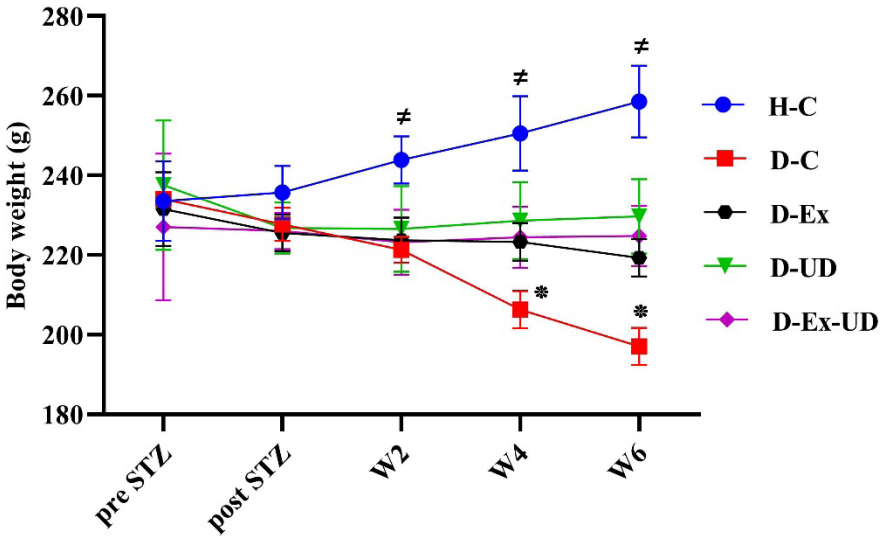

FIGURE 2. Changes in body weight during the study. Pre-STZ: represents baseline measurements obtained at the end of the treadmill familiarization period before diabetes induction. Post-STZ: indicates measurements taken after confirmation of diabetes induction. W2, W4, and W6 correspond to 2, 4, and 6 weeks after the start of interventions (endurance exercise and/or Urtica dioica extract), respectively. Each point represents Mean±SD for 10 rats in each group.
≠ Significant difference between H-C group with other groups (P<0.001), * Significant difference between D-C group with other groups (P<0.001). Healthy-Control (H-C), Diabetes-Control (D-C), Diabetes-Exercise (D-Ex), Diabetes-Urtica Dioica (D-UD), and Diabetes-Exercise-Urtica Dioica (D-Ex-UD).

STZ injection, especially at weeks 4 (W4) and 6 (W6) (P=0.001). In addition, at the end of the sixth week, no significant difference in BW was observed between the diabetic intervention groups, including the Ex, UD, and Ex-UD combination (P>0.05). Overall, intra-group trends indicate that endurance exercise and UD treatment attenuated diabetes-induced weight loss (Fig. 2).

The Effects of Ex and UD Interventions on Blood glucose (BG) in Diabetic Rats

The study's findings revealed that at the commencement of the study, the mean BG of the rats was 105±6.58 mg/dL. At 48 hours after the induction of diabetes by intraperitoneal injection of STZ, the BG in the diabetic groups increased significantly and reached 498.07±42.06 mg/dL. This increase was significantly different from the H-C group, demonstrating the successful induction of diabetes (F=140.25, P=0.001). Mauchly's test indicated violation of the sphericity assumption for the time factor (W=0.208, $\chi^2(9)=68.11$, P<0.001); therefore, Greenhouse–Geisser correction was applied. Repeated measures ANOVA demonstrated a highly significant main effect of time on BG (F(2.484, 111.758)=2139.543, P=0.001), a significant effect of group (F(4,45)=1479.821, P=0.001), and a significant time×group interaction (F(9.934, 111.758)=158.591, P=0.001), indicating different BG change patterns among the groups over time. However, post hoc comparisons indicated that EX and UD, both individually and in combination, significantly reduced BG levels in diabetic rats. This reduction began in the second week (combination treatment group) and continued to the fourth week (single treatment groups) and continued until the end of the experiment. By the end of the sixth week, the intervention groups showed a significant reduction in BG (F=720.61, P=0.001) compared with the D-C group (P=0.001). Furthermore, compared with the D-UD group, BG levels were significantly lower in the D-Ex-UD (P = 0.001) and D-Ex (P = 0.01) groups (Fig. 3). These findings underscore the remarkable effectiveness of these interventions in modulating diabetes-induced hyperglycemia.

The Effects of Ex and UD Interventions on TLR-4 Levels in Diabetic Rats

As shown in Figure 4, a significant difference was observed between the protein levels of TLR-4 of the different groups (one-way ANOVA, F(4,10)=88.21, P=0.0001). As such, streptozotocin-induced diabetes significantly elevated TLR-4 protein levels in the heart

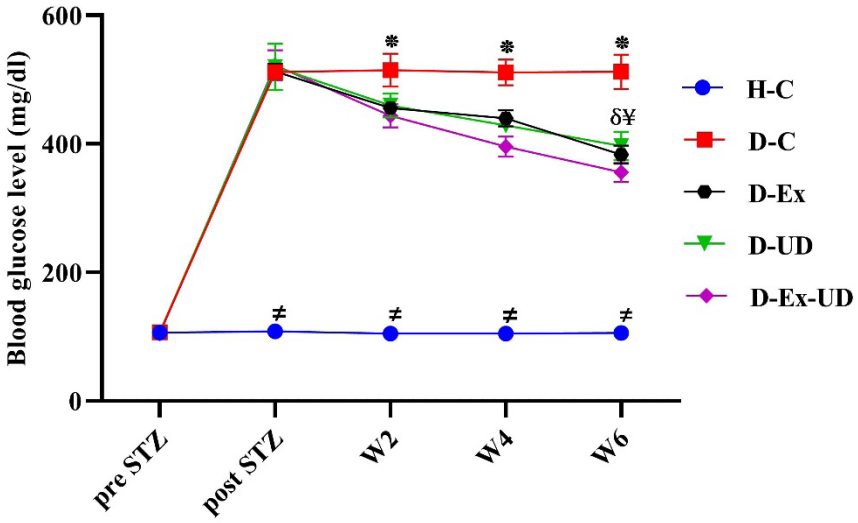

FIGURE 3. Changes in blood glucose during the study. Pre-STZ: represents baseline measurements obtained at the end of the treadmill familiarization period before diabetes induction. Post-STZ: indicates measurements taken after confirmation of diabetes induction. W2, W4, and W6 correspond to 2, 4, and 6 weeks after the start of interventions (endurance exercise and/or Urtica dioica extract), respectively. Each point represents Mean ± SD for 10 rats in each group.
≠ Significant difference between H-C group with other groups (P<0.001), * Significant difference between D-C group with other groups (P<0.001), ¥ Significant difference between D-Ex-UD group with D-UD group (P<0.001), δ Significant difference between D-Ex group with D-UD group (P<0.01). Healthy-Control (H-C), Diabetes-Control (D-C), Diabetes-Exercise (D-Ex), Diabetes-Urtica Dioica (D-UD), and Diabetes-Exercise-Urtica Dioica (D-Ex-UD).

tissue of rats in the D-C compared to the H-C group (P=0.0001). However, in diabetic rats, six weeks of Ex and/or UD treatment significantly lowered TLR-4 levels in the cardiac tissue of the D-UD, D-Ex, and D-Ex-UD groups when compared with the **D-C** group (P=0.0001). Additionally, UD treatment produced a greater decrease in TLR-4 levels than exercise alone, as shown by the comparison between D-UD and D-Ex groups (P=0.02). However, the most significant effectiveness was in the D-Ex-UD group, which showed a significant difference compared to the D-Ex group (P=0.001). On the other hand, no significant difference was observed between the D-Ex-UD and H-C groups (P=0.062), as well as between the D-Ex-UD and D-UD groups (P=0.328) (Fig. 4).

The Effects of Ex and UD Interventions on TNF-α and IL-10 Levels in Diabetic Rats

In this section, analysis using one-way ANOVA indicated significant differences in cardiac tissue TNF-α (F(4,45)=53.01, P=0.0001) and IL-10 (F(4,45)=14.61, P=0.0001) levels among the rat groups. STZ-induced diabetes significantly increased TNF-α and decreased IL-10 in the D-C group. Administration of either EX or UD independently led to a significant decline in TNF-α levels and an elevation in IL-10 levels (p<0.05). However, the most excellent modulation of these cytokines was observed in the combined group (Ex + UD). In this group, TNF-α levels were reduced more markedly than in the Ex-only group (P=0.0001) and UD-only group (P=0.028) (Fig. 5A). Additionally, the increase in IL-10 in the combination group was significant compared to the Ex-only group (P=0.012). However, it was not significant compared to that in the UD-only group (P=0.209) (Fig. 5B). Notably, no significant difference in TNF-α and IL-10 levels was observed between the H-C group and the D-Ex, D-UD, and D-Ex-UD groups in the cardiac tissue of the rats. Moreover, the IL-10 levels did not differ significantly among the D-Ex and D-UD groups (P > 0.05).

On the other hand, as shown in Figure 6, a significant difference was observed in the IL-10/TNF-α ratio in different groups (one-way ANOVA, F(4,45)=58.34, P=0.0001). This ratio was significantly reduced in the D-C group compared to the H-C group (P=0.0001), indicating a disruption of cytokine balance. However, the combined Ex and UD group (D-Ex-UD) exhibited the highest IL-10/TNF-α ratio, which was significantly

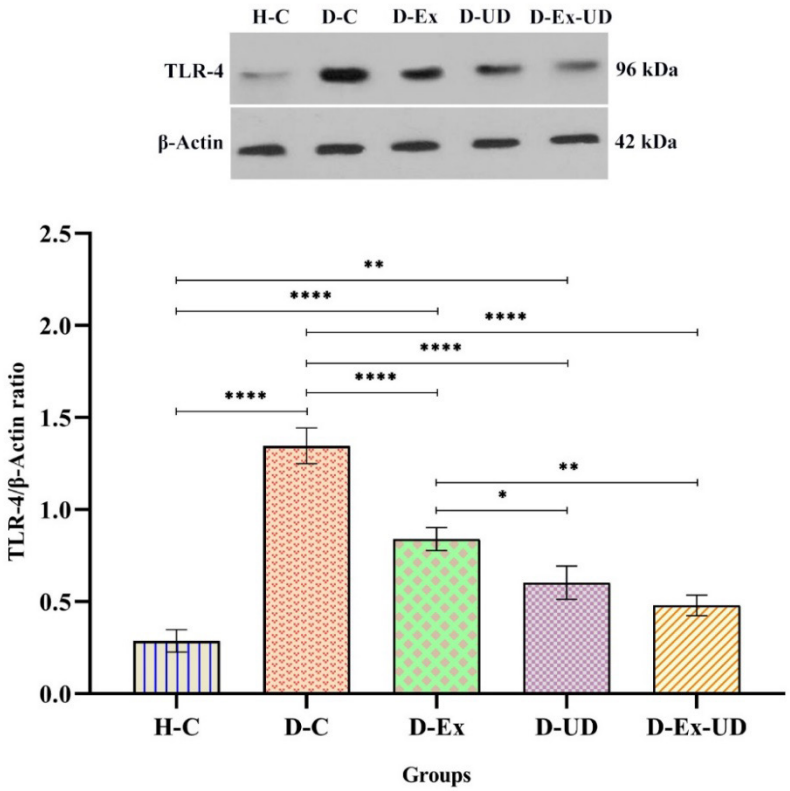

FIGURE 4. TLR-4 protein levels in the cardiac tissue of different groups. Western blot bands of TLR-4 protein and the reference protein β-Actin are shown above the graph. * Significant difference in level (P<0.05). ** Significant difference in level (P<0.01). **** Significant difference in level (P<0.0001). Healthy-Control (H-C), Diabetes-Control (D-C), Diabetes-Exercise (D-Ex), Diabetes-Urtica Dioica (D-UD), and Diabetes-Exercise-Urtica Dioica (D-Ex-UD).

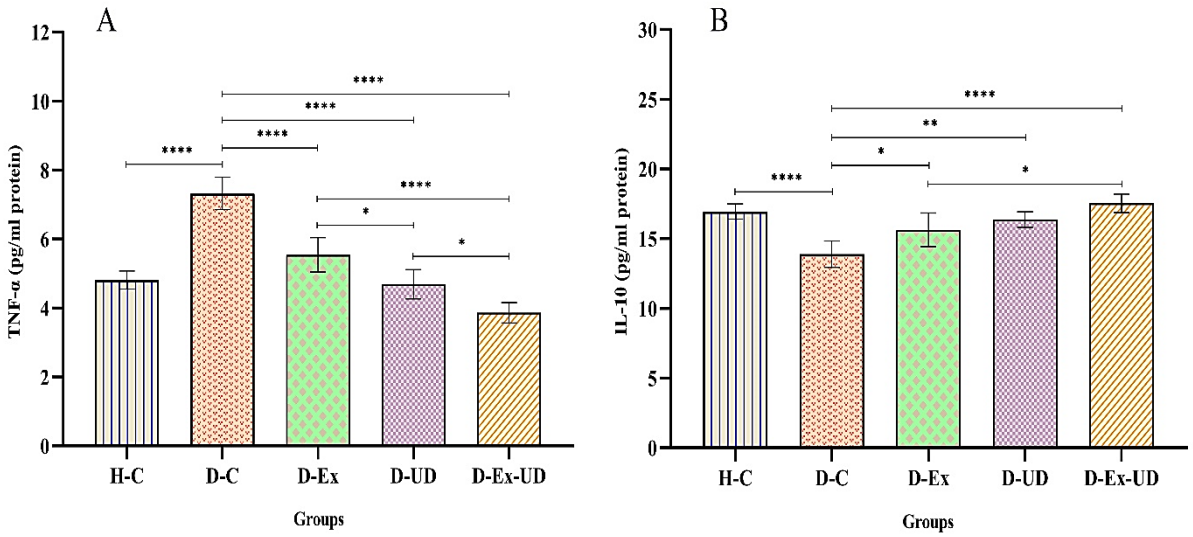

FIGURE 5. (A) Changes in the TNF-α levels in cardiac tissue of different groups. (B) Changes in the IL-10 levels in cardiac tissue of different groups. All data are presented in Mean ± SD. * Significant difference in level (P<0.05). ** Significant difference in level (P<0.01). **** Significant difference in level (P<0.0001). Healthy-Control (H-C), Diabetes-Control (D-C), Diabetes-Exercise (D-Ex), Diabetes-Urtica Dioica (D-UD), and Diabetes-Exercise-Urtica Dioica (D-Ex-UD).

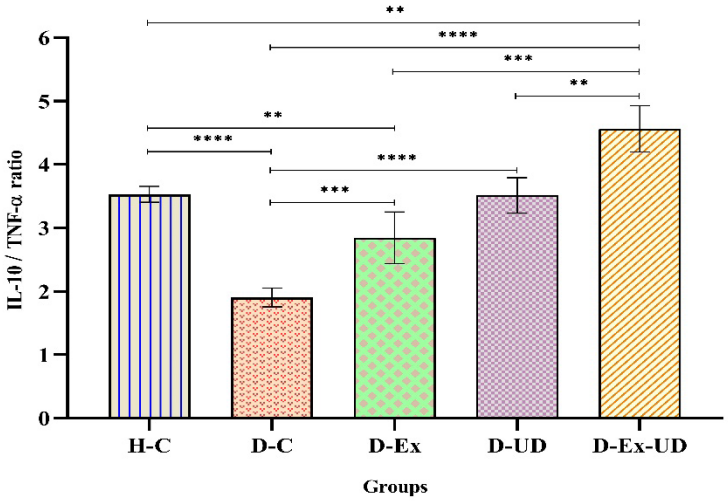

FIGURE 5. (A) Changes in the TNF-α levels in cardiac tissue of different groups. (B) Changes in the IL-10 levels in cardiac tissue of different groups. All data are presented in Mean ± SD. * Significant difference in level (P<0.05). ** Significant difference in level (P<0.01). **** Significant difference in level (P<0.0001). Healthy-Control (H-C), Diabetes-Control (D-C), Diabetes-Exercise (D-Ex), Diabetes-Urtica Dioica (D-UD), and Diabetes-Exercise-Urtica Dioica (D-Ex-UD).

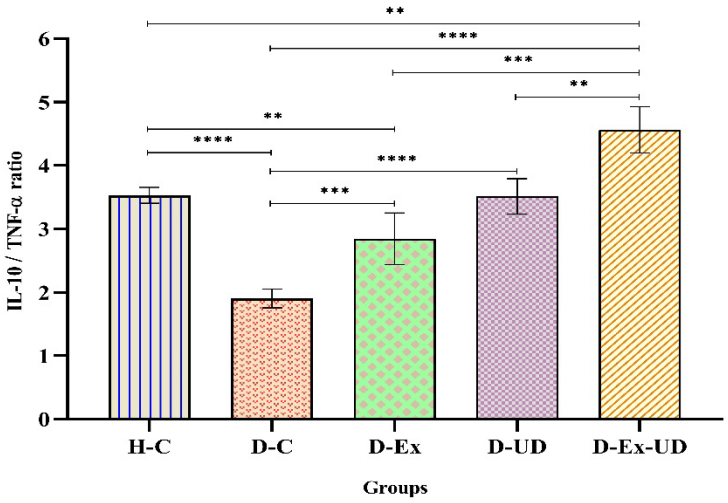

FIGURE 6. Changes in the IL-10/TNF-a ratio in the cardiac tissue of different groups. All data are presented in Mean ± SD. ** Significant difference in level (P<0.01). *** Significant difference in level (P<0.001). **** Significant difference in level (P<0.0001). Healthy-Control (H-C), Diabetes-Control (D-C), Diabetes-Exercise (D-Ex), Diabetes-Urtica Dioica (D-UD), and Diabetes-Exercise-Urtica Dioica (D-Ex-UD).

greater than that of the single-therapy groups (Ex or UD alone) (Fig. 6). This finding indicates that Ex and/or UD are capable of improving the IL-10/TNF-α inflammatory profile in diabetic cardiac tissue, even restoring or surpassing the levels of these inflammatory factors to those seen in the healthy group.

Discussion

Diabetes, characterized by insulin deficiency and impaired cellular glucose uptake, adversely affects body weight, blood glucose regulation, and the inflammatory status of cardiac tissue (dos Santos Haber et al., 2023; Keshvari et al., 2020). In the present study, we found that induction of diabetes in rats significantly reduced

BW, led to a severe increase in BG, and dysregulated the inflammatory status of cardiac tissue (increased TLR4 and TNF-α, decreased IL-10). However, endurance exercise and UD extract, either alone or in combination, significantly ameliorated these metabolic and inflammatory disorders. To the best of our knowledge, this is the first study to evaluate the combined effects of endurance exercise and Urtica dioica extract on metabolic and inflammatory alterations in the cardiac tissue of STZ-induced diabetic rats, highlighting the potential of multi-targeted therapeutic strategies. As an initial finding, we observed a significant increase in BG after STZ injection, confirming the successful induction of diabetes. Next, we aimed to investigate the impact of Ex and UD on BG levels. The results demonstrated that both endurance exercise (Ex) and UD extract significantly reduced blood glucose levels. This reduction began earlier in the combination therapy groups (from week two) and from week four in the single-therapy groups. Additionally, following diabetes induction, a decrease in BW was observed in rats two weeks after STZ injection, persisting until the sixth week. However, Ex and/or UD significantly helped maintain BW in diabetic rats. The treated groups experienced less weight loss versus the diabetic control group, particularly at weeks 4 and 6. These results underscore the beneficial role of Ex and/or UD in regulating blood glucose and mitigating diabetes-related weight reduction, consistent with findings from earlier studies. For instance, Wu et al. (2025) showed that 8 weeks of endurance training improved body weight, blood sugar, and insulin sensitivity in diabetic rats (Wu et al., 2025). Also, Ranjbar et al. (2019) reported that nettle aqueous extract and endurance training (alone or in combination) helped reduce blood glucose and improve body weight and cause tissue changes in the pancreas, liver, and heart of diabetic rats (Ranjbari et al., 2019). This evidence highlights the therapeutic potential of these interventions in controlling diabetes and its complications.

In diabetes, especially type 1 diabetes, insulin deficiency is the primary cause of weight loss and hyperglycemia. Insulin is an anabolic hormone that increases protein synthesis and inhibits lipolysis. In the absence of sufficient insulin, protein and fat catabolism for energy is increased glucose uptake by cells is impaired. These processes collectively lead to metabolic disorders, hyperglycemia, and unwanted weight loss in patients with diabetes (Kalhan 2009). In this study, the D-C group experienced the greatest weight loss and increased BG. This result is likely due to the lack of insulin compensation and reduced access of cells to glucose and amino acids, which leads to impaired cellular biosynthesis and metabolism. Our findings indicate that the co-administration of Ex and UD can effectively control BG and manage BW in diabetic subjects. These effects are likely mediated through activation of the AMPK signaling pathway, which subsequently increases the expression and activity of the GLUT4 protein in muscle. This process increases glucose uptake by muscles and reduces BG. Additionally, by inhibiting protein and fat catabolism, this compound helps maintain muscle mass and stabilize BW (Afzalpoura et al., 2016; Korani et al., 2017). UD leaves, rich in flavonoids and polyphenols such as scopoletin, rutin, esculetin, and quercetin, exhibit hypoglycemic properties (Rahmati et al., 2021). This effect is exerted through mechanisms such as stimulation of glucose production and breakdown in the liver (glycogenesis and glycolysis), increased insulin secretion from the pancreas, and reduced intestinal glucose absorption (by inhibiting alpha-amylase and alpha-glucosidase enzymes) (Kamaei and Moghadamnia 2019). It is worth noting that in the present study, dried UD leaf extract was used, which confirms and extends previous results on the antidiabetic properties of UD. The most significant reduction in BG in the combined group (Ex and UD) indicates a synergistic or additive effect of these two interventions. It appears that the distinct mechanisms of Ex (increased glucose absorption) and UD (stimulation of insulin secretion and reduced intestinal absorption) reinforce each other, contributing to more optimal BG control.

Another important outcome of this study was the notable improvement in cardiac inflammation observed after Ex and UD extract treatment. During the six-week intervention period, the applied strategies led to a downregulation of pro-inflammatory mediators TLR-4 and TNF-α, while concurrently promoting an elevation in the anti-inflammatory cytokine IL-10. Specifically, the combined group (Ex and UD) showed the greatest improvement in the IL-10/TNF-α ratio and inflammation control. This study revealed that both UD extract and Ex, whether applied independently or together, demonstrated therapeutic potential in attenuating the progression of DCM. These findings agree with previ-

ous studies that have demonstrated the anti-inflammatory properties of exercise and/or Urtica dioica through their modulatory effects on the TLR4/NF-κB pathway and the reduction of systemic inflammation. For instance, Parent et al. (2024) (Parente et al., 2024) and Rahmati et al. (2021) (Rahmati et al., 2021) have also reported reductions in inflammatory factors following UD consumption and Ex training. Similarly, Wu et al. (2014) showed reductions in TLR4 and NF-κB levels after moderate-intensity endurance exercise (Wu et al., 2014). Taken together, the results indicate that both UD extract and endurance exercise can effectively mitigate cardiac inflammation and alleviate DCM progression via several mechanisms, particularly through suppressing TLR4 and its downstream mediators. The observed reduction in TLR4 and TNF-α levels, and increase in IL-10 production induced by endurance exercise training can be attributed to several mechanisms. This effect is multifaceted: Firstly, by improving metabolic control (reducing hyperglycemia and dyslipidemia), exercise reduces the production of endogenous TLR4 ligands such as free fatty acids, AGEs, and DAMPs, which act as inflammatory "danger signals" (D'Haese et al., 2023; Kostrzewa-Nowak et al., 2020). Second, this reduction in danger signals inhibits TLR4 activation, which in turn attenuates pro-inflammatory pathways such as NF-κB and leads to the suppression of TNF-α (Fuentes-Antrás et al., 2014). In summary, endurance exercise both directly modulates TLR4 signaling pathways and indirectly reduces inflammatory stimuli, all contributing to the suppression of pro-inflammatory cytokine production. On the other hand, moderate-intensity endurance exercise significantly contributes to inflammation reduction by alleviating oxidative stress and enhancing antioxidant defenses. This finding has previously been shown in a study (Ghaffari et al., 2023). This reduction in oxidative stress inhibits TLR4 activity and TNF-α production, making the cellular environment less prone to chronic inflammation. Subsequently, this anti-inflammatory environment and the suppression ofNF-κB activate the anti-inflammatory PI3K/AKT/mTOR pathway, leading to increased production of IL-10, which in turn can modulate TLR4 levels (Rosa et al., 2011; Sayyad et al., 2022). In addition, exercise contributes to this process by shifting the phenotype of macrophages toward the M2 (anti-inflammatory) type, which are the main producers of IL-10 (Zhenyu et al., 2023). Consequently, endurance exercise acts as an important non-pharmacological and cardioprotective agent in diabetic individuals by reducing inflammatory factors and increasing anti-inflammatory ones.

Moreover, the present findings demonstrated that UD extract significantly reduced the levels of TLR4 and TNF-α in cardiac tissue, while conversely increasing IL-10 levels. These findings are biologically plausible, as UD has proven anti-inflammatory and antioxidant properties (Rahmati et al., 2021). In this context, a previous study demonstrated that UD extract can inhibit NF-κB activation, a crucial downstream pathway of TLR4 that plays a significant role in TNF-α gene transcription (Parente et al., 2024). Urtica dioica has broad antioxidant and anti-inflammatory properties due to its diverse compounds, including flavonoids (quercetin, kaempferol, scopoletin, apigenin, rutin, catechin, and myristicin) and other phenolic and sterol compounds (such as β-sitosterol, carvacrol, chlorogenic acid, gallic acid, and scopaltin) (El Haouari and Rosado 2019; Rahmati et al., 2021). Among these, quercetin, rutin, myristicin, kaempferol, and scopoletin play prominent roles in diabetes prevention and cardioprotection against inflammation. Specifically, quercetin, the most abundant flavonoid in UD (Rahmati et al., 2021), exhibits its potent anti-inflammatory effects by downregulating the TLR4/NF-κB/TNF-α signaling pathway. However, the precise cellular mechanisms of these compounds in protecting the heart against cardiomyopathy still require further investigation (Zhao et al., 2021). The observed cardioprotective and anti-inflammatory effects of Urtica dioica in diabetic rats are most likely attributable to its bioactive compounds. These compounds probably minimize inflammation by interfering with TLR4 signaling pathways (either by modulating upstream activators or by directly affecting downstream proteins) and by reducing the overexpression of TLR4. Although a direct reduction in TLR4 levels by UD has not been definitively demonstrated, its potent anti-inflammatory and antioxidant properties support this effect. UD helps reduce inflammation by inhibiting NF-κB (a primary outcome of TLR4 activation) and reducing oxidative stress (a major stimulator of TLR4 and NF-κB) (El Haouari and Rosado 2019; Fuentes-Antrás et al., 2014). The hydro-alcoholic extract of Urtica dioica and endurance exercise, with its anti-inflammatory, antioxidant, and immune-modulating properties, contributes to a favor-

able cytokine profile in the body. This results in an elevated IL-10/TNF-α ratio, thereby modulating immune responses and attenuating chronic inflammation. This study also demonstrated a significant increase in this ratio within the combined Ex and UD group, indicating favorable modulation of the immune cell phenotype. The findings suggest that the combined intervention promotes the macrophage polarization toward the M2 anti-inflammatory phenotype within the cardiac tissue of diabetic rats (dos Santos Haber et al., 2023; Zhenyu et al., 2023). One limitation of the present study is its relatively short duration. While a six-week period was sufficient to investigate changes in inflammatory markers, longer-term studies are needed to assess sustained effects on cardiac remodeling and chronic function in DCM. Although TLR-4, TNF-α, and IL-10 are considered key indicators of myocardial inflammation, other molecular factors also contribute to the pathogenesis of diabetes-induced cardiac inflammation. These factors include downstream signaling molecules of the TLR-4 pathway such as MyD88 and NF-κB; additional inflammatory cytokines including IL-1β and IL-6; and markers of oxidative stress, as well as activation of the NLRP3 inflammasome. Evaluation of these pathways in future studies may provide a more comprehensive understanding of the protective mechanisms of endurance exercise and extract of Urtica dioica in diabetic cardiomyopathy.

Conclusion

This study demonstrated that the co-administration of endurance exercise and hydro-alcoholic extract of Urtica dioica consumption exerts more favorable effects on metabolic control and myocardial inflammation in STZ-induced diabetic rats compared with either intervention alone. This combined intervention enhanced the anti-inflammatory response through modulation of TLR-4 levels and improvement of the inflammatory profile, particularly by increasing the IL-10/TNF-α ratio. These findings suggest a potential additive or co-administration effect, likely resulting from the interaction of complementary mechanisms of endurance exercise and the direct anti-inflammatory compounds present in extract of Urtica dioica that effectively target TLR-4-related inflammatory pathways.

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Conflict of interest

The authors declare that no conflict of interest in relation to the publication of this article.

Ethics approval

All animal handling procedures in this research were carried out in compliance with the guidelines of the National Institutes of Health (NIH, NO. 85–23, revised 1996) and were approved by the Research Ethics Committee of Lorestan University (IR: LU.ECRA.2019.17).

References

Ademolu A B. Sulfonylureas induced hypoglycemia in diabetics-a. International Journal of Diabetes and Endocrinology. Special Issue: Hypoglycemia in Diabetes 2019; 4: 108-112. https://doi.org/10.11648/j.ijde.20190404.14

Afzalpoura M E, Yousefib M R, Eivaric S H A, Ilbeigid S. Changes in blood insulin resistance, GLUT4 & AMPK after continuous and interval aerobic training in normal and diabetic rats. Journal of Applied Pharmaceutical Science 2016; 6: 076-081. https://doi.org/10.7324/JAPS.2016.60911

An J N, Yoo K D, Hwang J H, Kim H L, Kim S H, Yang S H, et al. Circulating tumour necrosis factor receptors 1 and 2 predict contrast-induced nephropathy and progressive renal dysfunction: A prospective cohort study. Nephrology 2015; 20: 552-559. https://doi.org/10.1111/nep.12448

Barry J C, Shakibakho S, Durrer C, Simtchouk S, Jawanda K K, Cheung S T, et al. Hyporesponsiveness to the anti-inflammatory action of interleukin-10 in type 2 diabetes. Scientific Reports 2016; 6: 21244. https://doi.org/10.1038/srep21244

Bostani M, Rahmati M, Mard S A. The effect of endurance training on levels of LINC complex proteins in skeletal muscle fibers of STZ-induced diabetic rats. Scientific Reports 2020; 10: 8738. https://doi.org/10.1038/s41598-020-65793-5

Byun E-B, Yang M-S, Choi H-G, Sung N-Y, Song D-S, Sin S-J, et al. Quercetin negatively regulates TLR4 signaling induced by lipopolysaccharide through Tollip expression. Biochemical and Biophysical Research Communications 2013; 431: 698-705. https://doi.org/10.1016/j.

bbrc.2013.01.056

Chehri A, Yarani R, Yousefi Z, Novin Bahador T, Shakouri S K, Ostadrahimi A, et al. Anti-diabetic potential of Urtica Dioica: current knowledge and future direction. Journal of Diabetes & Metabolic Disorders 2022: 1-10. https://doi.org/10.1007/s40200-021-00942-9

Choi RaYeong C R, Ham JuRi H J, Lee HaeIn L H, Cho HyunWook C H, Choi MyungSook C M, Park SeokKyu P S, et al. Scopoletin supplementation ameliorates steatosis and inflammation in diabetic mice. Phytotherapy Research 2017; 31:1795-1804. https://doi.org/10.1002/ptr.5925

D'Haese S, Verboven M, Evens L, Deluyker D, Lambrichts I, Eijnde B, et al. Moderate-and high-intensity endurance training alleviate diabetes-induced cardiac dysfunction in rats. Nutrients 2023; 15: 3950. https://doi.org/10.3390/nu15183950

dos Santos Haber J F, Barbalho S M, Sgarbi J A, de Argollo Haber R S, de Labio R W, Laurindo L F, et al. The relationship between type 1 diabetes mellitus, TNF-α, and IL-10 gene expression. Biomedicines 2023; 11: 1120. https://doi.org/10.3390/biomedicines11041120

El Haouari M, Rosado J A. Phytochemical, anti-diabetic and cardiovascular properties of Urtica dioica L.(Urticaceae): a review. Mini Reviews in Medicinal Chemistry 2019; 19: 63-71. https://doi.org/10.2174/1389557518666180924121528

Fuentes-Antrás J, Ioan A, Tunon J, Egido J, Lorenzo O. Activation of toll-like receptors and inflammasome complexes in the diabetic cardiomyopathy-associated inflammation. International Journal of Endocrinology 2014; 2014: 847827. https://doi.org/10.1155/2014/847827

Ghaffari G, Tofighi A, Rahmati M, Tolouei Azar J. The effect of one period of endurance training and consumption of hydroalcoholic extract of urtica dioica on oxidative stress indices of heart tissue in STZ-induced diabetic rats. Journal of Ilam University of Medical Sciences 2023; 31: 15-30.

Huang K, Luo X, Liao B, Li G, Feng J. Insights into SGLT2 inhibitor treatment of diabetic cardiomyopathy: focus on the mechanisms. Cardiovascular Diabetology 2023; 22: 86. https://doi.org/10.1186/s12933-023-01816-5

Kalhan S C. Fatty acids, insulin resistance, and protein metabolism. The Journal of Clinical Endocrinology and Metabolism 2009; 94: 2725-2727. https://doi.org/10.1210/jc.2009-1235

Kamaei L, Moghadamnia D. Comparison of antidiabetic effects of aqueous extract of the leaves and fruits of avicennia marina in streptozotocin-induced diabetic male rats. Iranian Journal of Toxicology 2019; 13: 7-12. https://doi.org/10.32598/IJT.13.2.575.1

Keshvari M, Rahmati M, Mirnasouri R, Chehelcheraghi F. Effects of endurance exercise and Urtica dioica on the functional, histological and molecular aspects of the hippocampus in STZ-Induced diabetic rats. Journal of Ethnopharmacology 2020; 256: 112801. https://doi.org/10.1016/j.jep.2020.112801

Korani B, Mirzapour A, Moghadamnia A A, Khafri S, Neamati N, Parsian H. The effect of urtica dioica hydro-alcoholic extract on glycemic index and AMP-activated protein kinase levels in diabetic patients: a randomized single-blind clinical trial. Iranian Red Crescent Medical Journal 2017; 19: e40572. https://doi.org/10.5812/ircmj.40572

Kostrzewa-Nowak D, Ciechanowicz A, Clark J S, Nowak R. Damage-associated molecular patterns and Th-cell-related cytokines released after progressive effort. Journal of Clinical Medicine 2020; 9: 876. https://doi.org/10.3390/jcm9030876

Morshed M, Alam J, Das M, Haque A, Ali L, Rokeya B. Antidiabetic and anti-inflammatory activity of Utica dioica leaves on stz induced type 1 diabetic model rats. International Journal of Pharmaceutical Sciences and Research 2011; 2: 1182.

Parente R, Paiva-Santos A C, Cabral C, Costa G. Comprehensive review of Urtica dioica L.(Urticaceae) phytochemistry and anti-inflammatory properties. Phytochemistry Reviews 2024: 1-38. https://doi.org/10.1007/s11101-024-09980-6

Patel S S, Gupta S, Udayabanu M. Urtica dioica modulates hippocampal insulin signaling and recognition memory deficit in streptozotocin induced diabetic mice. Metabolic Brain Disease 2016; 31: 601-611. https://doi.org/10.1007/s11011-016-9791-4

Rahmati M, Gharakhanlou R, Movahedin M, Mowla S J, Khazani A, Fouladvand M, et al. Treadmill training modifies KIF5B motor protein in the STZ-induced diabetic rat spinal cord and sciatic nerve. Archives of Iranian Medicine 2015; 18: 101.

Rahmati M, Kazemi A. Various exercise intensities differentially regulate GAP-43 and CAP-1 expression in the rat hippocampus. Gene 2019; 692: 185-194. https://doi.org/10.1016/j.gene.2019.01.013

Rahmati M, Keshvari M, Mirnasouri R, Chehelcheraghi F. Exercise and Urtica dioica extract ameliorate hippocampal insulin signaling, oxidative stress, neuroinflammation, and cognitive function in STZ-induced diabetic rats. Biomedicine & Pharmacotherapy 2021; 139: 111577. https://doi.

org/10.1016/j.biopha.2021.111577

Rajabi A, Akbar Nezhad Gharehlo A, Madadizadeh E, Basereh A, Khoramipoor K, Pirani H, et al. The effect of 12 weeks of aerobic exercise training with or without saffron supplementation on diabetes-specific markers and inflammation in women with type 2 diabetes: A randomized double-blind placebo-controlled trial. European Journal of Sport Science 2024; 24: 899-906. https://doi.org/10.1002/ejsc.12125

Ranjbari A, Salamat K, Akbarzadeh S, Khamisipour G. The effect of aqueous extract of nettle and endurance training on serum CRP, blood glucose, body weight, and changes in pancreatic, liver and heart tissues of STZ-diabetic rats. 2019.

Rosa J C, Lira F S, Eguchi R, Pimentel G D, Venâncio D P, Cunha C A, et al. Exhaustive exercise increases inflammatory response via toll like receptor-4 and NF-κBp65 pathway in rat adipose tissue. Journal of Cellular Physiology 2011; 226: 1604-1607. https://doi.org/10.1002/jcp.22490

Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Research and Clinical Practice 2019; 157: 107843. https://doi.org/10.1016/j.diabres.2019.107843

Samadian Z, Azar J T, Aghdam H R, Tofighi A, Razi M. Moderate-intensity exercise training in association with insulin promotes heat shock proteins 70 and 90 expressions in testicular tissue of experimental type 1 diabetes. Cell Journal (Yakhteh) 2021; 23: 708.

Sayyad M, Sheikhani H, Moqaddasi M, Jowhari A. The effect of 8 weeks of high-intensity interval water training on liver TLR-4 protein and serum TNF-α in male rats with non-alcoholic steatohepatitis by high-fat diet. Journal of Applied Health Studies in Sport Physiology 2022; 9: 125-135.

Wu X-D, Zeng K, Liu W-L, Gao Y-G, Gong C-S, Zhang C-X, et al. Effect of aerobic exercise on miRNA-TLR4 signaling in atherosclerosis. International Journal of Sports Medicine 2014; 35: 344-350. https://doi.org/10.1055/s-0033-1349075

Wu Y-W, Wu C-Y, Lin F, Wu J-Y. Exercise training benefits pancreatic islet by modulating the insulin-like growth factor 1/phosphatidylinositol 3-kinase/protein kinase B pathway. World Journal of Diabetes 2025; 16: 101447. https://doi.org/10.4239/wjd.v16.i5.101447

Yaturu S, Rains J, Jain S K. Relationship of elevated osteoprotegerin with insulin resistance, CRP, and TNF-α levels in men with type 2 diabetes. Cytokine 2008; 44: 168-171. https://doi.org/10.1016/j.cyto.2008.07.471

Zhao B, Zhang Q, Liang X, Xie J, Sun Q. Quercetin reduces inflammation in a rat model of diabetic peripheral neuropathy by regulating the TLR4/MyD88/NF-κB signalling pathway. European Journal of Pharmacology 2021; 912: 174607. https://doi.org/10.1016/j.ejphar.2021.174607

Zhenyu L, Ying W, Zhuang T, Yongchao X, Kim J. Exercise-mediated macrophage polarization modulates the targeted therapeutic effect of NAFLD: a review. Physical Activity and Nutrition 2023; 27: 10. https://doi.org/10.20463/pan.2023.0023