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The synergistic effects of glycyrrhizin and swimming exercise on CXCL12/CXCR4 gene

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Abstract

Background: This study aimed to investigate the modulating effects of two interventions, glycyrrhizin and swimming exercise, on the expression of CXCR4 and CXCL12 genes in the blood and heart tissue of diabetic rat models.

Methods: A total of 55 male Wistar rats were purchased, of which 44 were subcutaneously injected with streptozotocin between the ears. Four days post-injection, blood glucose levels were assessed. Rats exhibiting levels above 250 mg/dL were classified as diabetic and subsequently randomized into four groups of 11 animals each (Diabetic control, diabetic + glycyrrhizin, diabetic + swimming training, and diabetic + swimming training + glycyrrhizin). The healthy control group consisted of 11 rats. Two groups of diabetic rats were treated with glycyrrhizin. The core experimental protocol involved swimming training, which was implemented in two of the experimental groups. Glycyrrhizin was dissolved in 0.9% saline with sterile distilled water and administered by gavage at a dose of 120 mg/kg on average, transferred to the stomach of the animals every night. The main experimental protocol consisted of eight weeks of swimming training, which was performed in two experimental groups. The swimming training time started from 25 minutes with 7 liters of water in the adaptation period and increased to 60 minutes of activity in 17 liters of water in the eighth week. After eight weeks, the rats were anesthetized with ketamine and xylazine before blood collection, and then blood samples were taken from their hearts. Data analysis was performed using two-way analysis of variance and Tukey's test.

Results: A marked reduction in the expression levels of CXCR4 and CXCL12 was observed in the groups subjected to exercise and glycyrrhizin supplementation, indicating the potential of these interventions in modulating inflammatory signaling pathways (P-Value <0.05). The greatest reduction in CXCR4 (49.77%) and CXCL12 (68.19%) was observed in the combined exercise + glycyrrhizin group, indicating a stronger effect of the combined therapy than the single treatments.

Conclusion: Swimming training combined with glycyrrhizin supplementation significantly downregulated the expression of CXCR4 and CXCL12 genes and demonstrated potent antioxidant and anti-inflammatory effects, contributing effectively to the modulation and prevention of diabetes.

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Highlights

What is current knowledge?

The CXCR4 and CXCL12 genes play a crucial role in regulating glucose metabolism and inflammatory responses in diabetes. Glycyrrhizin, an active compound derived from licorice root, has anti-inflammatory properties, while swimming exercise increases insulin sensitivity and glucose utilization.

What is new here?

The combination of glycyrrhizin and swimming exercise significantly reduced the expression levels of CXCR4 and CXCL12 genes compared to the diabetic control group, indicating a synergistic therapeutic effect.

Introduction

Diabetes is a complex metabolic disorder characterized by chronic hyperglycemia and impaired metabolism of carbohydrates, lipids, and proteins. The disease is associated with activation of various inflammatory pathways, oxidative stress, and endothelial dysfunction (1). Among these, the CXCL12/CXCR4 axis plays a critical role in inflammatory responses, tissue repair, and glucose homeostasis. CXCL12, a chemokine, and its receptor CXCR4 are involved in key processes such as cell migration, angiogenesis, proliferation, and survival, and are important modulators of immune and inflammatory responses (2). The activity of this axis is altered in diabetic conditions, and CXCL12 and CXCR4 gene expression changes accordingly (3).

In some cases, increased CXCL12 expression is associated with oxidative stress and NF-κB-related inflammatory pathways, whereas decreased CXCR4 expression may impair cellular responsiveness to repair signals (4). It can also affect receptor activity and modulate CXCL12/CXCR4 expression. Glycyrrhizin, a natural triterpenoid compound extracted from the root of Glycyrrhiza glabra (Licorice), possesses a wide range of pharmacological properties, including anti-inflammatory, antioxidant, and immunomodulatory effects. Its molecular mechanism of action is the inhibition of high mobility group box 1 (HMGB1). By binding directly to HMGB1, glycyrrhizin prevents its interaction with Toll-like receptors and the receptor for advanced glycation end-products (RAGE), thereby suppressing downstream inflammatory cascades such as NF-κB and MAPK pathways (5). In diabetic conditions, glycyrrhizin has shown promising effects in reducing oxidative stress, improving insulin sensitivity, and modulating the CXCL12/CXCR4 signaling axis. Its antioxidant capacity is mediated through the activation of the Nrf2 pathway, which enhances the expression of cytoprotective enzymes. Glycyrrhizin influences key signaling molecules such as AMPK and PI3K/Akt, contributing to improved metabolic homeostasis and reduced organ damage (6). Preclinical studies further support its therapeutic potential, particularly when combined with lifestyle interventions such as regular physical activity (7). Non-pharmacological approaches such as glycyrrhizin and regular exercise have been considered potential strategies for managing diabetes and reducing its complications (8). Glycyrrhizin may affect multiple signaling pathways, including the CXCL12/CXCR4 axis (9),

while swimming exercise, as a form of aerobic exercise, may also help improve metabolic control and reduce inflammation in diabetic conditions (10).

Swimming exercise has been reported to have effects at the molecular level. These changes are associated with the activation of several signaling pathways, including AMPK, PGC-1α, and Nrf2 (11). Evidence suggests that regular physical activity can affect CXCL12 and CXCR4 expression in some tissues. One of the major challenges in this field is the association between changes in CXCL12/CXCR4 expression with improvements in metabolic parameters and reduction of diabetic complications (12). Changes in the expression of these genes are important for determining the effective dose of glycyrrhizin and the optimal intensity and duration of swimming exercise to modulate CXCL12 and CXCR4 expression in diabetic conditions (13).

The CXCL12/CXCR4 axis plays a key role in inflammatory responses, cell migration, tissue repair, and regulation of glucose metabolism (14). Both glycyrrhizin and exercise can influence diabetes-related parameters; however, their combined effect on CXCL12/CXCR4 gene expression in the context of diabetes is not fully understood. This study aims to address this gap (14). These findings offer valuable insights into the molecular basis of non-pharmacological interventions for diabetes, potentially guiding the refinement of glycyrrhizin dosing and exercise intensity to maximize therapeutic efficacy.

Wistar rats are widely used as a standard animal model in diabetes research due to their physiological and metabolic similarities to humans and their high sensitivity to streptozotocin (STZ)-induced diabetes. This model allows for precise investigation of molecular mechanisms, including the expression of inflammatory genes such as CXCL12 and CXCR4. Their appropriate body size, high tolerance to exercise interventions, and ease of blood and tissue sampling make Wistar rats a suitable and reliable choice for preclinical studies.

Methods

All experimental procedures and animal work were conducted under the protocols of the Research Ethics System and the Declaration of Helsinki (Ethics code: IR.IAU.VARAMIN.REC.1402.02). The study sample consisted of 55 male Wistar rats (Weight: 180-220 g). A one-week adaptation period to the environment was considered under standard controlled conditions of light (12 hours of light and 12 hours of darkness), temperature (22 ± 3 °C), and humidity (About 50%). During this period, the rodents had free access to food in the form of pellets and water. Then, 44 rats were made diabetic by STZ injection (Sigma Aldrich). Four days after the injection, rats with blood sugar levels above 250 mg/dL were randomly divided into the following groups: diabetic control, diabetic + swimming exercise, diabetic + glycyrrhizin, diabetic + swimming exercise + glycyrrhizin. Eleven healthy rats were placed in the healthy control group.

To reduce bias and ensure uniform distribution of confounding factors, the animals were randomly assigned to different experimental groups using a random number table in Excel software after confirming the inclusion criteria (Including blood sugar above 250 mg/dL for diabetic groups).

The training program included swimming in a special tank (A plastic swimming tank with dimensions of 70 × 90 × 150 cm). The training program was conducted on specific days between 5 and 7 pm (Table 1).

Fifty-five male Wistar rats were obtained from a certified laboratory animal supplier. The animals were housed under controlled environmental conditions with a temperature of 22 ± 2 °C and relative humidity of 55 ± 5%. A 12-hour light/dark cycle was maintained (e.g., lights on from 7:00 AM to 7:00 PM).

The rats underwent a one-week acclimatization period prior to the experiment to minimize stress associated with transportation and new environmental conditions. During this period, they had free access to standard laboratory chow and water ad libitum. The animals were housed in durable plastic cages, with an average of 3-4 rats per cage to avoid overcrowding and reduce stress. Handling and transportation of the rats throughout the study were conducted carefully to minimize stress. The rats were transferred individually using clean gloves or specialized transport boxes to avoid direct contact. All equipment and cages were thoroughly disinfected between uses to prevent cross-contamination.

Two groups of the diabetic rats were given glycyrrhizin at a dose of 120 mg every day at 12:00–12:30 PM orally via gastric tubes. The blood sugar levels of the rats were measured before STZ injection on days 0, 7, 14, 21, and 35. The rats were anesthetized with ether before blood samples were taken.

Table 1. Eight-week swimming training program for diabetic rats (15)

Exercise variables	Week	Time (s)	Temperature (°C)	Water volume (lit)	Water speed L/M
Feminization	1	25	32-30	4	7
Main program	2	30		4	
	3	35		5	
	4	40		5	11
	5	45		6	
	6	50		6	
	7	55		7	15
	8	60		7	

Preparation and use of glycyrrhizin

In this study, glycyrrhizin was manually extracted from the dried root of the licorice plant (*Glycyrrhiza glabra*). Licorice roots were obtained from Marvdasht farms in Shiraz, and after washing and drying completely, they were powdered. For extraction, an aqueous-ethanolic solvent extraction method was used, where the plant powder was placed in a 70% ethanol solution at room temperature for 48 hours and stirred intermittently. After filtering and evaporating the solvent using a rotary evaporator, a concentrated extract was obtained. To separate the glycyrrhizin compound, a precipitation technique using acid and centrifugation was employed, and the final purity was confirmed by HPLC. The final dried powder was stored at 4 °C and dissolved in physiological saline before use. The approximate purity of glycyrrhizin obtained by this method was estimated to be about 85-90%. The average dose of glycyrrhizin was determined to be 120 mg/kg. The pH of the solution was maintained in the range of 7.2-7.4. This solution was administered to the rats every day at 12:00-12:30 PM for eight weeks. After a certain volume of glycyrrhizin solution was prepared based on the animal's body weight (In mg/kg), the rats were gently restrained. Then, a round-tipped gavage needle was inserted through the oral cavity and passed along the esophagus to the stomach. Once the needle was in place, the solution was slowly injected using a syringe. After injection, the needle was slowly withdrawn (16).

Method of taking blood samples from the heart

To collect blood samples from the rats, the animals were first completely anesthetized by intraperitoneal injection of a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg). A 1-3 mL syringe with a 23- or 25-gauge needle was then gently inserted into the heart through the fourth or fifth intercostal space. The syringe plunger was gently withdrawn to collect blood without collapsing the cardiac vessels (Equivalent to 6 mL). After collection, the blood samples were immediately placed in tubes containing anticoagulant (EDTA) for plasma.

Examination of CXCR4 and CXCL12 gene expression

RNA extraction from the blood samples was performed using the QIAamp RNA Blood Mini Kit (Qiagen, product code 52304). The concentration and purity of the RNA were assessed using spectrophotometry (Nanodrop 2000, Thermo Scientific). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a cDNA synthesis kit according to the manufacturer's instructions. First, 1.5 mL of whole blood was mixed with 7.5 mL of EL buffer, and after lysis of red blood cells, leukocytes were collected by centrifugation (400 g for 10 min). Then, the cells were lysed with 600 µL of RLT buffer containing β-mercaptoethanol (10 µL/mL) and mixed with 600 µL of 70% ethanol. The resulting solution was transferred to a QIAamp column and, after washing with RW1 and RPE buffers, RNA was extracted with 40 µL of RNase-free water. cDNA synthesis was performed using the QuantiTect reverse transcription kit (Qiagen) with 1 µg of RNA.

Quantitative real-time PCR (qPCR) was performed using the ABI 7500 Fast Real-Time PCR System with SYBR Green master mix. Each 20 µL reaction contained 10 µL of SYBR Green master mix, 0.5 µM of each forward and reverse primer, 2 µL of cDNA template, and nuclease-free water.

The qPCR cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 58-60 °C for 30 s, and extension at 72 °C for 30 s. At the end of amplification, melting curve analysis was performed to confirm the specificity of the PCR products.

All experiments (RNA extraction, cDNA synthesis, and real-time PCR reactions) were performed in triplicate (Biological replicates) for each sample. Moreover, each PCR reaction was performed in triplicate (Technical replicates) to ensure accuracy and reliability of the data. Relative gene expression levels were calculated using the 2^(-ΔΔCt) method with GAPDH or β-actin as internal controls.

Total RNA was extracted from the samples, and its purity was assessed using spectrophotometry (Nanodrop 2000, Thermo Scientific). cDNA was synthesized from 1 µg of total RNA using a cDNA synthesis kit according to the manufacturer's instructions.

The thermal program consisted of initial activation (50 °C for 2 min and 95 °C for 10 min), followed by 40 cycles of denaturation (95 °C, 15 s) and annealing/extension (60 °C, 1 min) (Table 2).

Statistical analysis

Statistical analysis of data was performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). First, the normality of data distribution was checked using the Shapiro–Wilk test. Two-way analysis of variance was used to compare the means of groups, and if a significant difference was observed, Tukey's post hoc test was used to examine the difference between the groups. Gene expression results presented are from three replicates (Mean ± SEM) of independent experiments. The mean mRNA expression levels of the treated samples were compared with the mean of the control samples (=1) and analyzed (P<0.05).

Table 2. Sequences of forward and reverse primers of the genes of interest for the real-time PCR reaction

Genes	Primer sequences	Size (bp)
GAPDH	Forward: 5'- 'AGTGCCAGCCTCGTCTCATA-3'	147
	Reverse: 5'- 'GATGGTGATGGGTTTCCCGT-3'	
CXCR4	Forward: 5'-' GACTGGCATAGTCGGCAATG-3'	134
	Reverse: 5'- 'AGAAGGGGAGTGTGATGACAAA-3'	
CXCL12	Forward: 5'- 'CTGCCGATTCTTTGAGAGCC-3'	272
	Reverse: 5'- 'TTCGGGTCAATGCACACTTG-3'	

Results

To better understand the effect of training, physiological data such as body weight, insulin levels, and cardiac function were measured (Table 3).

The mean, standard deviation, and percentage of changes in CXCR4 and CXCL12 were recorded in different research groups (Each group with 11 rats) (Table 4).

The two independent factors in two-way ANOVA were glycyrrhizin supplementation and swimming training. The interaction effects between these two factors were explicitly tested and reported in the results section (Table 5). Before analysis, the assumptions of the ANOVA were confirmed. The normality of the residuals was assessed using the Shapiro-Wilk test, and the homogeneity of variances was checked using the Levene test. All assumptions were met, and no violations were observed.

Table 3. Some physiological characteristics of the rats

Variables	Healthy control	Diabetic	Diabetic + Glycyrrhizin	Diabetic + Exercise	Diabetic + Glycyrrhizin + Exercise
	Mean ± SD				
Body weight (g)	280 ± 15	220 ± 18	230 ± 15	235 ± 14	245 ± 12
Insulin level (ng/mL)	1.2 ± 0.25	0.5 ± 0.15	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.2
Blood glucose (mg/dL)	95 ± 10	350 ± 30	280 ± 25	240 ± 22	220 ± 20
Heart rate (bpm)	380 ± 20	350 ± 25	360 ± 20	370 ± 19	375 ± 18
Systolic blood pressure (mmHg)	115 ± 10	130 ± 12	125 ± 10	120 ± 10	118 ± 9

Table 4. Descriptive and analytic report of research variables

Groups / Variable	H	D	D + E	D + G	D + G + E	F	P-Value
	Mean ± SD						
CXCR4	1.0000±0.16990	2.1850±0.33611	1.3025±0.27621	1.9900±0.89394	1.0975±0.61646	4.148	0.019*
CXCL12	1.0000±0.23833	3.7175±0.83024	3.5100±0.48298	2.8725±1.04672	1.1825±0.36854	14.966	0.000*

Table 5. Results of the two-way analysis of variance test for CXCR4 and the effect size of each independent variable

Source	Type III sum of squares	df	Mean square	F	P-Value	Partial eta squared
Corrected model	1.828[a]	3	0.609	1.393	0.281	0.207
Intercept	40.903	1	40.903	93.489	0.000	0.854
Swimming	0.042	1	0.042	0.097	0.760	0.006
Glycyrrhizin	1.598	1	1.598	3.653	0.074	0.186
Swimming × Glycyrrhizin	0.415	1	0.415	0.948	0.345	0.056
Error	7.000	16	0.438	-	-	-
Total	54.733	20	-	-	-	-
Corrected total	8.829	19	-	-	-	-
a. R squared = 0.207 (Adjusted R squared = 0.058)						

The results of two-way ANOVA and Tukey's post hoc test showed that the CXCR4 gene was significantly reduced in the diabetes induction + glycyrrhizin + swimming group compared to the diabetes induction group (P-Value = 0.345). The difference between the healthy control group and the diabetes induction + glycyrrhizin + swimming group was not significant (P-Value = 1.000). The large p-value (P-Value = 1.000) for the comparison between the healthy control group and the diabetes induction + glycyrrhizin + swimming group in Tukey's post hoc test indicates that the average CXCR4 expression level in these two groups was nearly identical (Figure 1). Based on the results of two-way ANOVA, the difference between the diabetes induction group and the diabetes induction + glycyrrhizin group had the most significant effect (ŋ = 0.186, P-Value = 0.074, F = 3.653). According to the table, the largest effect after consuming glycyrrhizin is related to the intervention of both independent variables (ŋ = 0.056, P-Value = 0.345), followed by the smaller effect size of swimming training (ŋ = 0.006, P-Value = .760) (Figure 1).

CXCR4 gene expression in the diabetic group (D) was significantly increased, reaching approximately 2.18-fold compared to the healthy control group (HC). This increase indicates a potential role of CXCR4 in the inflammatory processes. In the glycyrrhizin-treated groups (D+G), swimming training (S+D), and combination treatment (D+S+G), a significant decrease in the expression of this gene was observed. In particular, the combination treatment showed the greatest decrease, bringing the gene expression value to 1.09, which is very close to the healthy group. Both interventions, especially in combination, were able to effectively inhibit CXCR4 expression and thus may be effective in reducing inflammatory responses in diabetic conditions.

The results of two-way ANOVA and Tukey's post hoc test showed that the CXCL12 gene was significantly downregulated in the diabetes induction + glycyrrhizin + swimming group compared to the diabetes induction group (P-Value = 0.018). The difference between the healthy control group and the diabetes induction + glycyrrhizin + swimming group was not significant (P-Value = 1.000). The large p-value (P-Value = 1.000) for the comparison between the healthy control group and the diabetes induction + glycyrrhizin + swimming group in Tukey's post hoc test indicated that the average CXCL12 expression level in these two groups was nearly identical (Figure 2). Based on the results of two-way ANOVA, the difference between the diabetes induction group and the diabetes induction + glycyrrhizin + swimming training group was significant (ŋ = 0.301, P-Value = 0.018, F = 6.897). According to Table 6, the highest effect was in the group with the intervention of both swimming and glycyrrhizin (ŋ = 0.301, P-Value = 0.018). Swimming training had a lesser effect (ŋ = 0.149, P-Value = 0.113), and the least effect was observed in the glycyrrhizin group (ŋ = 0.015, P-Value = 0.625).

Exercise or glycyrrhizin alone had a modulating effect, but the combination of exercise and glycyrrhizin caused a strong decrease in expression (Down to 1.18-fold), which was statistically significantly different from the other groups. These results indicate that the combined intervention is more effective in reducing the negative effects of diabetes on gene expression (Table 7).

It can be concluded that a period of swimming training and glycyrrhizin supplementation, both separately and in combination, reduced CXCR4 and CXCL12 gene expression in the diabetic-induced rats.

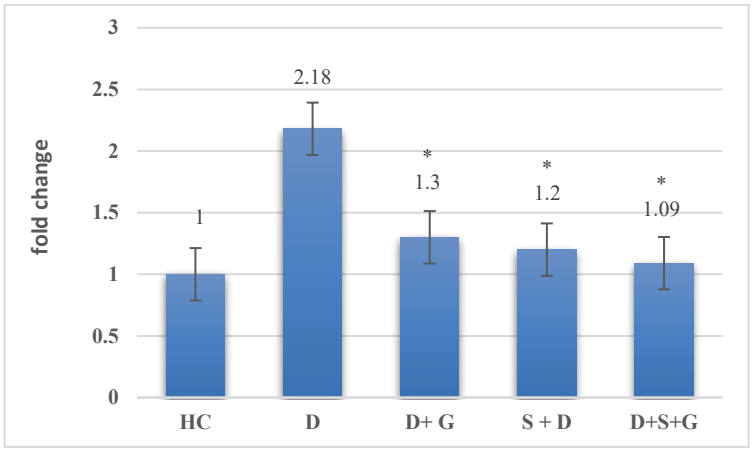

Figure 1. Comparison of mean CXCR4 mRNA

HC (Healthy Control); D (Diabetic); D+G (Diabetic + Glycyrrhizin); S+D (Swimming + Diabetic); D+S+G (Diabetic + Swimming + Glycyrrhizin)
*: Statistical significance (P-Value < 0.05) compared to the diabetic group

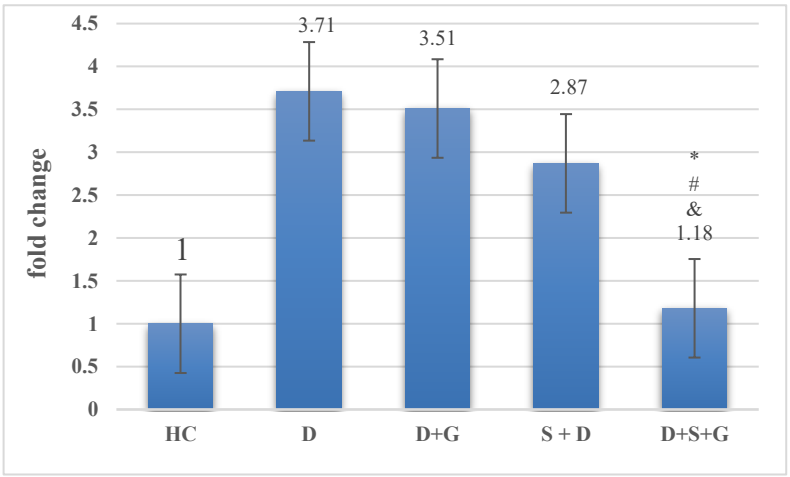

Figure 2. Comparison of mean CXCL12 mRNA

HC (Healthy Control); D (Diabetic); D+G (Diabetic + Glycyrrhizin); S+D (Swimming + Diabetic); D+S+G (Diabetic + Swimming + Glycyrrhizin)
*: Significant difference between the control group and the diabetic, Diabetic + Glycyrrhizin, and Diabetic + Swimming training groups
#: Significant difference between the diabetic group and the diabetic + Swimming training + Glycyrrhizin group
&: Significant difference between the diabetic + Glycyrrhizin and the diabetic + Glycyrrhizin + Swimming groups

Table 6. Results of the two-way analysis of variance test for CXCL12 and the effect size of each independent variable

Source	Type III sum of squares	df	Mean square	F	P-Value	Partial eta squared
Corrected model	11.700[a]	3	3.900	2.916	0.066	0.353
Intercept	112.550	1	112.550	84.141	0.000	0.840
Swimming	3.760	1	3.760	2.811	0.113	0.149
Glycyrrhizin	0.332	1	0.332	0.248	0.625	0.015
Swimming × glycyrrhizin	9.226	1	9.226	6.897	0.018	0.301
Error	21.402	16	1.338	-	-	-
Total	153.790	20	-	-	-	-
Corrected total	33.102	19	-	-	-	-
a. R squared = 0.353 (Adjusted R squared = 0.232)						

Table 7. Post hoc test results and P-Values for comparisons between each pair of groups for each gene

Variable	Groups	Tukey's statistic	P-Value
CXCR4	Healthy vs. Diabetic	5.17	0.043
CXCL12	Healthy vs. Diabetic	11.23	0.001
	Healthy vs. Diabetic + Exercise	7.74	0.001
	Healthy vs. Diabetic + Licorice	10.37	0.001
	Diabetic vs. Diabetic + Exercise + Licorice	10.48	0.001
	Diabetic + Exercise vs. Diabetic + Exercise + Licorice	6.98	0.001
	Diabetic + Licorice vs. Diabetic + Exercise + Licorice	9.62	0.001

Discussion

The study results were associated with a decrease in CXCL12 and CXCR4 gene expression, indicating a synergistic effect of the combined interventions of exercise and supplementation.

Effects of combined treatment

A possible mechanism is the synergy of the CXCL12/CXCR4 signaling axis and its pivotal role in the pathophysiology of diabetes. Under hyperglycemic conditions, CXCL12 (A chemokine) and its receptor CXCR4 usually cause increased inflammatory responses (9). Therefore, the combined treatment approach of glycyrrhizin (An active compound derived from licorice root) and swimming exercise has shown the potential to modulate the expression of these genes in a diabetic mouse model (17). Glycyrrhizin can directly affect the CXCL12/CXCR4 axis due to its potent anti-inflammatory and antioxidant properties. Research by Yang et al. (2023) demonstrated that the administration of glycyrrhizin in diabetic models significantly reduced CXCR4 expression (18). This effect is believed to occur through the inhibitory effect of glycyrrhizin on HMGB1, a damage-associated molecular pattern that activates CXCL12/CXCR4 signaling in hyperglycemic states (19). The resulting downregulation of CXCR4 limits inflammatory cell recruitment, thereby protecting against diabetes-induced organ damage (20). Furthermore, regular swimming exercise combined with glycyrrhizin supplementation appears to significantly modulate chemokine signaling, particularly by altering CXCL12 expression. This modulation is associated with improved glucose homeostasis and reduced systemic inflammation.

Potential mechanisms

The underlying mechanisms likely involve activation of the AMPK (AMP-activated protein kinase) pathway, which suppresses NF-κB-mediated transcription of proinflammatory chemokines such as CXCL12. In addition, swimming increases the bioavailability of nitric oxide, which has been shown to increase CXCR4 expression (21). Together, these interventions appear to normalize the hyperactive CXCL12/CXCR4 cascade. Reduction of CXCL12 and CXCR4 limits inflammation. Restoration of normal CXCL12 levels also helps reactivate stem cell functions that are often impaired in chronic diabetes (22). This dual intervention likely creates a regenerative microenvironment in contrast to the fibrotic and inflammatory environment (23).

Swimming exercise may also have effects through the PI3K/Akt signaling pathway (24) and downregulates CXCL12 expression. Glycyrrhizin may exert its regulatory effects through inhibition of the HIF-1α pathway. In diabetic conditions, hypoxia-induced vascular dysfunction leads to stabilization of HIF-1α, a potent transcriptional activator of CXCL12 (25). Furthermore, elevated cortisol levels, which are often observed in chronic metabolic stress, are associated with upregulation of CXCR4 in immune cells. Therefore, the reduction of cortisol through swimming exercise may contribute to the downregulation of CXCR4 expression (26). The combination of swimming exercise and glycyrrhizin supplementation also has significant regulatory effects on the Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway, which is involved in the regulation of antioxidant defense mechanisms. Increased Nrf2 activity can suppress NF-κB, a key transcriptional activator of CXCL12 and CXCR4, thereby explaining the synergistic effect of these two interventions in reducing the expression of inflammatory chemokines (27). Regular swimming training alters DNA methylation patterns in the CXCL12 promoter regions, potentially affecting its transcriptional activity. In parallel, glycyrrhizin inhibits histone deacetylase activity, leading to increased histone acetylation and greater accessibility of transcription factors to the CXCL12 and CXCR4 gene promoters (28). In addition, both interventions affect the expression of microRNAs (miRNAs) that post-transcriptionally regulate CXCL12/CXCR4. Specifically, swimming exercise has been associated with the upregulation of miRNAs that negatively regulate these genes, contributing to the suppression of their expression in diabetic conditions (29,30). Another novel mechanism is the regulation of autophagy. In diabetic conditions, impaired autophagy has been associated with increased CXCR4 expression. Glycyrrhizin, by modulating the autophagy pathway, may restore cellular homeostasis and thus indirectly reduce CXCL12/CXCR4 expression (31).

Comparison with previous studies

CXCL12 and CXCR4 genes, known for their pivotal roles in immune cell recruitment and inflammatory responses, are often upregulated in pathological conditions such as diabetes, indicating activation of proinflammatory signaling pathways. Yang et al. (2023) reported a significant decrease in CXCR4 expression in cancer after a similar therapeutic approach, suggesting the broader immunomodulatory potential of this intervention (18). Similarly, Pahlavani (2022) showed that regular swimming exercise increases Akt phosphorylation, which subsequently inactivates FOXO3a, a transcription factor known to upregulate CXCR4 expression (24). However, this study did not include any pharmaceutical or herbal supplements.

Werner et al. (2013) further confirmed the anti-inflammatory effects of specific plant compounds, showing that treatment in a model of chronic intestinal inflammation resulted in reduced expression of CXCL12 and CXCR4 in the intestinal mucosa (32). Similarly, Sadeghi et al. (2023) investigated the effect of medicinal plants on these genes, although exercise was not part of their protocol (33). In the context of metabolic diseases, Gaur et al. (2025) observed that metformin administration in models of type 2 diabetes significantly reduced CXCR4 expression, suggesting its role in balancing inflammation and cell survival (34). In contrast, Wang et al. (2022) found that diabetic mice treated with mesenchymal stem cells had increased expression of CXCL12 and CXCR4, and attributed these changes to the activation of repair pathways (35).

Furthermore, in a model of cerebral spinal ischemia, Rojin Sarallah et al. (2025) reported that injection of CXCL12-containing gel increased CXCR4 expression in neurons and glial cells, thereby promoting neuronal regeneration and improving motor recovery (36). Consistent with these findings, Li et al. (2016) showed that regular aerobic exercise in healthy mice increased CXCL12/CXCR4 expression, which was associated with increased neovascularization and improved muscle metabolism (22). However, not all increases in gene expression are necessarily pathological. Huang et al. (2025), studying cancer patients undergoing chemotherapy, observed increased expression levels of these genes in the absence of any supplementation (37).

Taken together, these findings suggest that while CXCL12 and CXCR4 are generally upregulated in response to inflammation or injury, their expression can be modulated in a context-dependent manner. In the present study, both swimming exercise and glycyrrhizin supplementation effectively reduced the expression of these genes in diabetic mice. This downregulation is likely mediated through reduced oxidative stress, suppression of NF-κB signaling, and subsequent inhibition of proinflammatory pathways.

Some limitations of the study should be noted. This study was conducted in an animal model, and generalization of the results to humans requires larger clinical studies. Only two types of interventions (Aerobic swimming and the herbal compound glycyrrhizin) were used, and examining other forms of exercise or anti-inflammatory drugs could provide a more comprehensive view. This study only examined the expression of CXCL12 and CXCR4 genes and did not examine other molecular pathways related to inflammation and oxidative stress, nor did it perform histopathological evaluations of heart or pancreatic tissue, which could provide direct evidence of the effects of the interventions on tissue structure. The duration of the intervention was also relatively limited. Only male rats were used.

It is recommended that clinical studies be conducted in diabetic patients. The association of these genes with other inflammatory and oxidative stress markers, such as TNF-α, IL-6, and NF-κB, and the combined effects of exercise training with other herbal or pharmaceutical supplements should be investigated. The use of more advanced molecular techniques, such as RNA sequencing or signaling pathway analysis, and histopathological assessments could also help to more precisely identify the mechanisms involved in the regulation of CXCL12/CXCR4 gene expression. The choice of female rats as the research sample may also reveal differences.

Conclusion

The findings of this study showed that aerobic swimming training and glycyrrhizin consumption, separately and in combination, significantly reduced the expression of CXCL12 and CXCR4 genes in the blood of the diabetic rats. This reduction may indicate a decrease in the activity of inflammatory pathways. The interventions examined in this study played a regulatory and protective role against cell damage caused by diabetes.

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Ethical statement

All animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Varamin Pishva Branch, Islamic Azad University, under the ethical approval code IR.IAU.VARAMIN.REC.1402.017. All efforts were made to minimize animal suffering and reduce the number of animals used.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

F.N. conceptualized and designed the study, while F.N. and Z.M. performed the experiments and collected the data. Both F.N. and Z.M. carried out the molecular analyses, as well as the statistical analyses and interpreted the results. F.N. wrote the initial draft of the manuscript. All authors reviewed, edited, and approved the final version of the manuscript.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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