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The role of aerobic training with the *Vaccinium Arctostaphylos* L. fruit hydro-alcoholic extract in the regulation of oxidative stress in the heart tissue of diabetic rats

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Abstract

Background: Cardiovascular disease is a significant contributor to mortality in diabetic patients. Despite the potential benefits of concomitant and complementary training in diabetic patients, there is a paucity of evidence in this area.

Aim: The objective of this study was to investigate the effect of aerobic training in conjunction with a hydroalcoholic extract of the fruit of Qaraqat on superoxide dismutase, glutathione peroxidase, total antioxidant capacity, and malondialdehyde in the heart tissue of male rats with type 2 diabetes.

Materials and Methods: 24 male rats aged 8 weeks were diabetic with a high-fat diet (60%) and streptozotocin injection. They were randomly divided into four groups: (1) aerobic training (AT), (2) supplementation (Sup), (3) Aerobic training+ supplement (AT+Sup), and (4) diabetes control (Con)**.** The training protocol included running on a treadmill at a speed of 5-24 m/min for 10-60 min, according to the principle of overload. Glucose, insulin, insulin resistance, and MDA, TAC, GPX, and SOD of heart tissue were measured. Statistical tests of two-way variance and post hoc Bonferroni were used using SPSS software at a significance level of $P<0.005$.

Results A significant decrease in glucose levels, insulin resistance, and MDA ($P<0.005$) was observed in the intervention groups following eight weeks of training and supplement intervention, in comparison to the Con group. Also, the amount of insulin in Sup and AT+Sup groups exhibited a notable decline in comparison to Con and AT groups ($P<0.005$), as well as a substantial increase in the levels of SOD ($P=0.001$) and GPx ($P<0.005$), and TAC ($P<0.005$) was observed.

Conclusion: Diabetes is associated with elevated MDA levels, which are reduced by aerobic training and supplementation. Furthermore, the administration of a training intervention and the utilization of a supplement have been demonstrated to elevate the levels of SOD, TAC, and GPx.

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1. Introduction

Diabetes is a metabolic disorder that is mainly associated with high blood sugar levels and cardiovascular complications, resulting in an increased risk of mortality [1]. High glucose levels in heart cells lead to the accumulation of reactive oxygen species in the heart's muscle cells, ultimately leading to the death of heart cells [2]. It has been reported that the strengthening of the antioxidant system or the administration of antioxidants can significantly reduce the severity of heart disease in diabetic patients with cardiovascular disorders [3].

Oxidative stress is a phenomenon that arises from an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues, and the inability of the biological system to detoxify these reactive products, ROS are produced as a byproduct of oxygen metabolism [4]. It is well-established that an increased production of ROS has the potential to both impair antioxidant mechanisms and cause irreversible oxidative damage to nucleic acids, DNA, membrane lipids, proteins, cell metabolism, message transmission, cell proliferation, and gene expression. Such damage has been linked to the development of various pathologies, including those associated with diabetes and cancer [5]. Furthermore, ROS activates the C-Jun N-terminal kinase (JNK) and NF-κB pathways, which disrupt the insulin signaling pathway and induce insulin resistance [6, 7].

Oxidative stress plays an important role in the development of cardiovascular complications associated with diabetes. Metabolic disorders of diabetes result in the excessive production of mitochondrial superoxide in endothelial cells of large and small vessels, as well as in the myocardium [8]. In the absence of pathological conditions, the body's antioxidant and defense systems are capable of scavenging free radicals in both enzymatic and non-enzymatic ways. However, certain factors, such as diabetes, can result in the production of large quantities of free radicals, thereby inducing oxidative stress [9]. Also, due to the antioxidant status of the body and the increase in the production of free radicals, malondialdehyde (MDA) is one of the most common byproducts of lipid peroxidation, and total antioxidant capacity (TAC) is among the indicators that are measured [10].

In the body, some antioxidant enzymes are responsible for the destruction of free radicals, including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). Each of these enzymes has a distinct mode of operation. SOD catalyzes the conversion of the superoxide anion radical into H_2O and O_2, while CAT catalyzes the conversion of hydrogen peroxide into water and oxygen. GPx converts hydrogen peroxide into water, while GR facilitates the regeneration of reduced glutathione (GSH) [11]. Additionally, glutathione-S-transferase functions as a secondary enzyme, facilitating the neutralization of toxins. This is achieved by establishing a link between ROS and GSH, or by neutralizing lipid peroxides, thereby neutralizing the effects of free radicals [12].

Aerobic training is one of the best non-pharmacological interventions for blood glucose control [13]. It has been demonstrated that regular physical activity and exercise increase the number of glucose transporter 4 (GLUT4) molecules and an enhancement in membrane permeability. Also, the reduction in glycogen concentration

in muscles that occurs during exercise persists for a considerable time after the cessation of the workout, due to the subsequent effort to rebuild glycogen stores. This results in a sustained reduction in blood sugar levels [14].

Kamr and Vinu (2024) showed that aerobic exercise significantly decreased blood sugar levels. The benefits of aerobic training for diabetic patients include the strengthening of the antioxidant system and the reduction of oxidative factors [15]. Exercise and physical activity can increase antioxidant enzymes such as SOD and GPX and reduce oxidation [10]. It can also be stated that regular and continuous aerobic training increases mitochondrial function through a decrease in ROS. Additionally, aerobic training improves the synthesis of nitric oxide and biogenic mitochondria. Therefore, it can be concluded that aerobic exercise is an effective method for reducing oxidative stress and can have beneficial effects for individuals with diabetes [13]. In this regard, Klarod et al. (2023) showed that exercise has beneficial effects on antioxidant balance [16]. Also, Naderi et al. (2015) demonstrated that exercise significantly reduced MDA levels and increased levels of SOD, GPX in the blood and heart tissue of diabetic rats [17]. Also, Akbarpour et al. (2023) after eight weeks of resistance training and TRX, a significant increase was observed in SOD and GPX levels, accompanied by a considerable reduction in MDA levels [18].

The utilization of medicinal plants in the treatment of diabetes represents a significant area of interest. One such plant is Qaraqat (*Vaccinium Arctostaphylos*), which contains a distinctive profile of phenolic acid and a range of phenolic compounds [19]. Additionally, Anthocyanins are present in abundance and have been demonstrated to possess a multitude of properties, including anti-diabetic [20], antioxidant, anticancer, and anti-inflammatory [21]. It can be posited that herbal supplements have the effect of lowering blood sugar levels [20].

Barut et al. (2019) reported that Qaraqat has antioxidant, anti-inflammatory, alpha-glucosidase inhibitor and protective effects for DNA. It can be therefore stated that Qaraqat is an effective medicinal plant for the prevention and treatment of inflammatory diseases and oxidative damage [22]. In addition, Shamilov et al. (2022) investigated the presence of phenolic compounds, which are known to possess neuroprotective and antioxidant properties, in the leaves of Qaraqat. They showed the presence of hydroxycinnamate and flavonoids in the leaves, with caffeoylquinic acid identified as the predominant metabolite. This compound has been demonstrated to enhance cerebral hemodynamics, mitigate necrosis and lip oxidative processes in brain tissue, and boost antioxidant enzyme activity [23].

Several studies have shown that aerobic training at a moderate intensity can enhance antioxidant enzyme activity in humans [24] and also in mice [25]. Aerobic training reduces oxidative damage caused by increased mitochondrial antioxidant enzymes and decreases oxygen flow in the respiratory chain [26]. Furthermore, the ingestion of Qaraqat has been demonstrated to possess antioxidant, anti-inflammatory, antioxidative, and antidiabetic properties, which collectively contribute to the reduction of blood sugar levels and the strengthening of the body [20, 21]. According to our searches, little research has been conducted in this area. To the best of our knowledge, no study has

investigated the interaction effect of Qaraqat supplementation and aerobic training on diabetes; Therefore, this study aimed to investigate the effect of aerobic training with hydroalcoholic extract of Qaraqat fruit on MDA, TAC, GPX, and SOD in heart tissue of type 2 diabetic male rats.

2. Materials and Methods

2.1. Experimental animal

The present study is an experimental study in the form of a multi-group single-agent post-test design with a control group, conducted at the Faculty of Veterinary Medicine of the University of Tabriz from April to August 2023. It is based on the regulations on the use of laboratory animals, as approved by the Ethics Committee of the University of Tabriz with the ID (IR. TABRIZU. REC.1402.037). In the present study, 24 male Wistar rats with an average weight of 200±20 gr and an age of 8 weeks were used in the laboratory animal breeding and maintenance center of the Faculty of Veterinary Medicine of Tabriz University. To ensure environmental compatibility, prevent stress, and modify physiological conditions, all mice were placed in a dedicated laboratory setting with specific environmental conditions. The implementation period of the protocol was conducted with groups of four mice in polyethylene cages at ambient temperatures of 20 to 22 °C, light to dark cycle of 12:12 hours, and humidity of 55 to 65%. During this period, rat food was provided in the form of standard open-access platforms.

2.2. Diabetes induction method

Following a two-week period during which insulin resistance was established, 12 rats were placed on a high-fat diet (HFD) comprising 20% protein, 20% carbohydrates, and 60% fat [27]. Subsequently, by injection into the theory of streptozotocin (STZ), a low-dose (35 mg/kg) sodium citrate buffer solution (pH= 4.5) was used alongside ice [28]. To induce experimental diabetes, after determining the weight and blood sugar of fasting mice were first determined. The injection site was then disinfected with alcohol, and the prepared solution of streptozotocin was injected intraperitoneally using insulin syringes. After the injection, the mice were transferred to the cage and fed with food and water. 72 hours after streptozotocin injection, diabetes induction was confirmed by fasting blood glucose (FBG) measurement, and animals with blood glucose higher than 300 mg/dl were considered diabetic rats [28].

2.3. Preparation of hydro-alcoholic extract of Qaraqat

First, pure ethanol was subjected to heating, after which the ethanol vapor was entirely captured and collected by the Cloninger device. The method employed enabled the collection of evaporated ethanol without the presence of Bittrex bitter poison. Then, ethanol was concentrated to a concentration of 70% and combined with a powdered plant material. After that, the resulting mixture was filtered and, the obtained liquid was subjected to a drying to procedure to produce a pure extract. Subsequently, the extracts were subjected to a drying process, after which they were filtered and concentrated using a rotary evaporator under reduced pressure at approximately 40°C. The filtered plant extracts were combined and stored at -20°C until the oxidative stress inhibition assay was conducted [29]. Gas and mass chromatography (MC/GC) was used to determine the compounds in the extract according to the relevant protocols [30]. The administration time was as follows: The plant extract was dissolved in 0.2 cc of normal

saline at a dose of 250 mg/kg of body weight and was administrated to mice by gavage on a daily basis. All procedures were carried out in the specialized laboratories of the Faculty of Veterinary Medicine at Tabriz University.

2.4. Experimental groups

A total of 24 rats were randomly divided into four groups (N=6) as follows: (1) Diabetic group with aerobic training (AT), high-fat food, and streptozotocin injection and training; (2) Diabetic group with supplement (Sup), high-fat food and streptozotocin injection, and Qaraqat supplementation without training; (3) Diabetic group with aerobic training and supplement (AT+Sup), high-fat food and streptozotocin injection, and Qaraqat supplementation and training; and (4) Diabetic control group (Con), high-fat food, and streptozotocin injection, without training and supplementation. The rats were subjected to aerobic training two weeks after the induction and maintenance of diabetes.

2.5. Aerobic training protocol

The Dia+AT and Dia+AT+Sup groups performed a training program on a treadmill for five days per week over a period of eight weeks (Table 1).

Table 1. Aerobic training protocol with overload principle

Week	Speed (m/min)	Time (min)	Slope
First	5-10	10-15	**10**
Second	10-14	20	**10**
Third	14-18	30	**10**
Fourth	18-24	40	**10**
Fifth / Sixth	18-24	50	**10**
Seventh/eighth	18-24	60	**10**

A five-minute warm-up and a five-minute cool-down period were incorporated at the commencement and conclusion of the training session, respectively. The intensity of the workout was deemed to be appropriate for a warm-up and cool-down at a speed of 50% [31]. To stimulate the mice to run, a mild electric shock with a very low voltage was used. The increase in speed and training time was achieved through the application of the overload principle.

2.6. Sample collection and tissue homogenization for heart tissue

To measure the study variables, the mice were anesthetized with a combination of ketamine (80 mg/kg body weight) and xylazine (10 mg/kg body weight) after 12 to 14 hours of fasting. This was done in accordance with ethical principles and by intraperitoneal injection. The mice were then operated on painlessly by trained specialists [32]. Initially, blood samples were taken directly from the heart with heparin-impregnated syringes and transferred to test tubes and serum in centrifuges with a rotation speed of 2500-3000 rpm for a period of 10-15 min. The isolated serum was stored in a freezer set to -80°C in order to measure glucose and insulin levels. Following the collection of blood samples, the entire heart tissue was removed and placed within a microtube containing liquid nitrogen. It was then stored in a minus-80 freezer until the time of molecular evaluation.

2.6.1. Assay superoxide dismutase (SOD)

To measure this variable, a commercially available Nasdox^{TM} SOD test kit manufactured by the company Nand Salamat (Iran) and bearing the catalog number (15032-Cat No: NS), was employed at a wavelength of 375 nm and with a sensitivity of (U/mg pr). The method is based on the inhibition of the pyrogallol autoxidation reaction. The compound is oxidized under normal conditions in the vicinity of air. By

determining the half-life of its self-oxidation at a certain concentration, the rate of the reaction can be calculated. In this reaction, the inhibition of the SOD auto-oxidation reaction is measured at a certain time by comparing a sample containing SOD with the concentration of SOD. All work steps have been carried out according to the kit manufacturer's instructions.

2.6.2. Assay glutathione peroxidase (GPx)

In order to achieve this objective, a commercial kit for measuring GPx enzyme activity, Nagpix™ manufactured by Nand Salamat (Iran) and bearing the catalog number (Cat No. 15082-No. NS), was employed. In general, the method of measuring this variable is based on the reduction of absorbance at the wavelength of 340 nm, with the resulting value expressed on the (U/mg pr) scale. GPx is responsible for the oxidation of glutathione to glutathione. The regeneration of glutathione oxide is contingent upon the presence of glutathione reductase, which is accompanied by the oxidation of NADPH to $NADP^+$ and the reduction of light absorption at a wavelength of 340 nm. All steps of the procedure were conducted in accordance with the instructions provided by the kit manufacturer.

2.6.3. Assays total antioxidant capacity (TAC)

The Naxifer™ kit manufactured by the company Nand Salamat (Iran) with the number (NS-15012) was used to measure this variable. In summary, this method is founded upon the capacity to reduce iron to ferric iron (FRAP) through a single electron transfer mechanism. The color change of the reaction was measured at 593 nm and the resulting standard graph was employed to obtain quantitative TAC. All procedures were conducted in accordance with the instructions provided by the kit manufacturer.

2.6.4. Assay malondialdehyde (MDA)

For this purpose, a commercial Nalondi™ lipid peroxidation test kit manufactured by the company Nand Salamat (Iran) with the number (Cat No. 15022-NS) was used. In summary, MDA reacts with thiobarbituric acid (TBA) at elevated temperatures to produce a pink colour, which is then measured calorimetrically at a wavelength of 530-540 nm. All procedures were conducted in accordance with the instructions provided by the kit manufacturer.

2.6.5. Glucose level measurement

The blood glucose concentration was determined using Clover Cheek (TD-4230) blood glucose test kit by glucose oxidase method. The nocturnal fasting glucose level was applied by drawing blood samples from the tail between the hours of 8:00 and 8:30 a.m. on one occasion (48 hours after the last training session). The concentration of insulin in the blood was determined using a specific ELISA kit for mice, produced by the Shanghai Cryostat Diabetic Company in China. The immunoassay method employed was based on the sandwich ELISA technique. The (HOMA-IR) index was used to calculate insulin resistance [33].

$$\text{HOMA-IR} = [\text{Glucose (mg/dl)} \times \text{insulin (mU/L)}] / 405$$

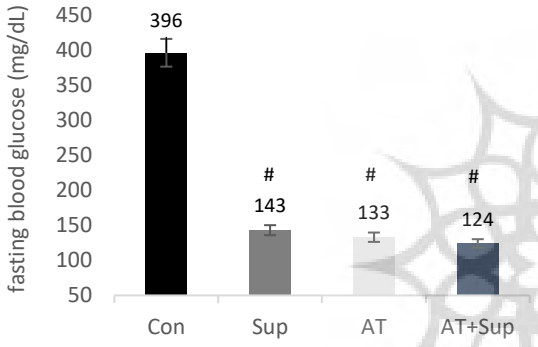
2.7. Statistical analyses

In order to ascertain the normality distribution of the data, the Shapiro-Wilk test and two-way ANOVA were used to evaluate the differences between the groups. The post-hoc Bonferroni test was used to observe the differences in the studied groups. The analysis was performed at the significant level of $P<0.005$ using SPSS software version 27.

3. Results

3.1. Fasting blood glucose (FBG)

A two-way analysis of variance showed that there was no statistically significant reduction in glucose levels observed in the AT group when compared to the Sup group (*P*=1.000). Bonferroni's post hoc test showed a significant decrease in glucose in all three intervention groups compared to the Con group (*P*=0.001). Nevertheless, no significant decrease was observed between the AT+Sup group compared to the AT and Sup groups (*P*>0.005).

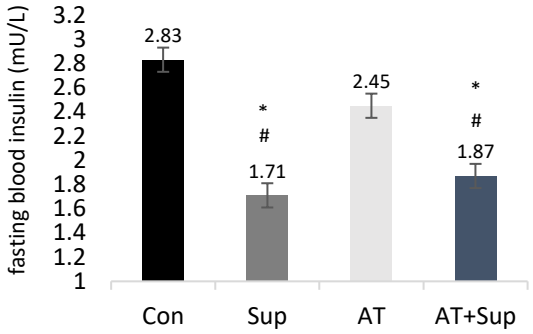


# *P*<0.05, significant difference from the Con group

Figure 1. Comparison of the serum FBG levels of the AT+Sup, AT, and Sup groups after the intervention with the Con group.

3.2. Fasting blood insulin

A two-way analysis of variance showed that fasting blood insulin (mU/L) in the Sup group had a significant decrease compared to the AT group (*P*=0.001). Bonferroni's post hoc test demonstrated that fasting blood insulin was significantly reduced in the Sup and the AT+Sup groups compared to the Con group (*P*=0.001). There was no significant difference between the AT and Con groups (*P*=0.060). Additionally, a notable decline was observed in the AT+Sup group in comparison with the AT group (*P*=0.001). Conversely, no considerable reduction was evident in the Sup group when contrasted with the AT+Sup group (*P*=1.000).



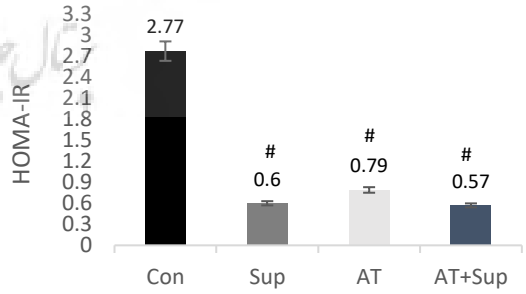
# *P*<0.05, significant difference from the Con group

* *P*<0.05, significant difference with AT group

Figure 2. Comparison of the fasting blood insulin levels of the AT+Sup, and Sup groups after the intervention with the Con group. and AT group with AT+Sup and Sup groups

3.3. Insulin resistance (HOMA-IR)

The two-way analysis of variance revealed that there was no statistically significant reduction in insulin resistance in the Sup group in comparison to the AT group (*P*=1.000). Bonferroni's Post hoc test showed a significant decrease in insulin resistance in all three intervention groups compared to the Con group (*P*=0.001). Nevertheless, no notable decline was observed between the AT+Sup group compared to the AT and Sup groups (*P*>0.005).

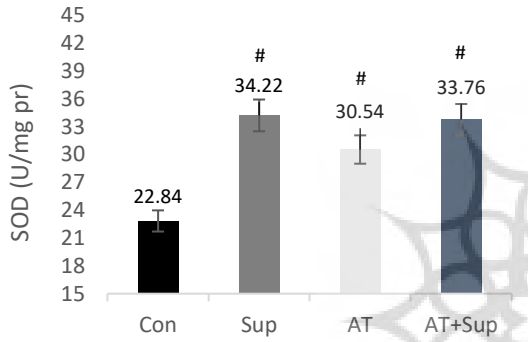


# *P*<0.05, significant difference from the Con group

Figure 3. Comparison of the insulin resistance levels of the AT+Sup, AT, and Sup groups after the intervention with the Con group.

3.4. Superoxide dismutase (SOD)

A two-way analysis of variance revealed a non-significant increase in the level of SOD in the Sup group relative to the AT group (*P*=1.000). Bonferroni's Post hoc test demonstrated that the level of SOD in all three intervention groups exhibited a significant increase compared to the Con group (*P*=0.001). However, no significant difference was observed between the AT+Sup group and the AT and Sup groups (*P*>0.005).

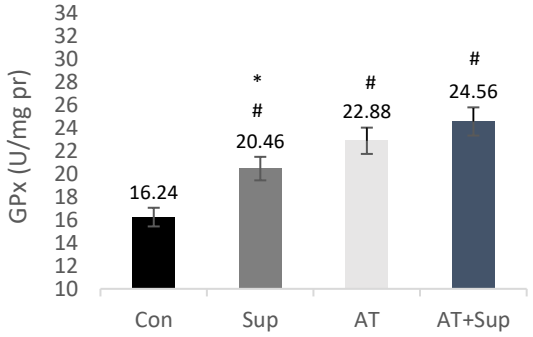


# *P*<0.05, significant difference from the Con group

Figure 4. Comparison of the superoxide dismutase levels of the AT+Sup, AT, and Sup groups after the intervention with the Con group

3.5. Glutathione peroxidases (GPx)

The two-way analysis of variance revealed an elevation in the level of GPx in the AT group relative to the Sup group; however, this increase was not statistically significant (*P*=0.413). Bonferroni's post hoc test demonstrated a notable rise in the level of GPx in the AT+Sup and AT groups. Furthermore, the AT+Sup group exhibited a statistically significant increase in GPx levels compared to the Con group (*P*=0.001). Additionally, the Sup group demonstrated a notable elevation in GPx levels compared to the Con group (*P*=0.022). When comparing the AT+Sup group to the Sup group, there was a significant difference in GPx levels (*P*=0.027). However, no statistically significant difference was observed between the AT and Sup groups (*P*>0.005).



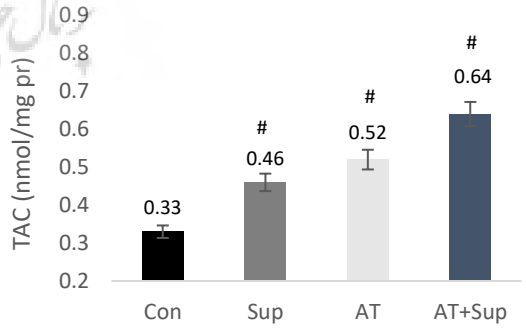
# *P*<0.05, significant difference from the Con group

* *P*<0.05, significant difference with AT+Sup group

Figure 5. Comparison of GPxs levels of the AT+Sup, AT, and Sup groups after the intervention with the Con group and Sup group with AT+Sup group

3.6. Total antioxidant capacity (TAC)

Two-way analysis of variance showed a non-significant increase in TAC level in the AT group compared to the Sup group (*P*=1.000). Bonferroni's post hoc test demonstrated a significant increase in TAC level in the AT+Sup, AT, and Sup groups (*P*<0.05) compared to the Con group. But there was no significant difference between the AT+Sup group compared to the AT and Sup groups (*P*<0.05).

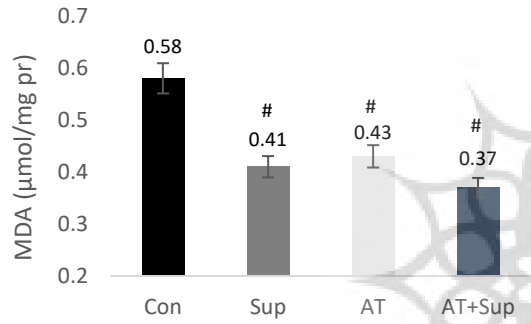


# *P*<0.05, significant difference from the Con group

Figure 6. Comparison of TAC levels of the AT+Sup, AT, and Sup groups after the intervention with the Con group.

3.7. Malondialdehyde (MDA)

The two-way analysis of variance demonstrated that the level of MDA in the Sup group did not exhibit a statistically significant reduction in comparison to the AT group (P=1.000). Bonferroni's post hoc test demonstrated that the level of MDA in all three intervention groups exhibited a statistically significant reduction in comparison to the Con group (P<0.05). Nevertheless, no statistically significant difference was observed between the AT, Sup, and AT+Sup groups (P>0.005).



# P<0.05, significant difference from the Con group

Figure 7. Comparison of the MDA levels of the AT+Sup, AT, and Sup groups after the intervention with the Con group

4. Discussion

The findings of the present study demonstrated that a period of eight weeks of diabetes resulted in a notable elevation in glucose levels and insulin resistance within the Con group, when compared to the other groups. This was observed to be reversed with eight weeks of aerobic training. Furthermore, significant differences were observed in the levels of SOD, MDA, TAC, and GPx between the intervention groups and the Con group.

The results showed that the MDA levels were elevated in the Con group relative to the AT, Sup, and AT+Sup groups. However, no statistically significant differences were observed between the three intervention groups with respect to the MDA variable. It seems that the presence of elevated MDA levels in diabetic patients is indicative of oxidative stress [34]. As the disease progresses, there is an increase in MDA levels, which indicates a high production of free radicals, lipid peroxidation, and cellular oxidative damage. This can contribute to the long-term complications associated with diabetes [35].

On the other hand, a reduction in MDA levels was observed in the AT group in comparison to the Con group. This finding was consistent with the results of Hooshmand et al. (2019) which also demonstrated that aerobic training reduces MDA levels in individuals with type 2 diabetes. It is evident that MDA is a prevalent byproduct of lipid peroxidation, which is the most important factor for measuring oxidative stress. Reducing this through aerobic training can be an important factor, and physical activity is beneficial for diabetic patients by reducing oxidative stress and maintaining the integrity of pancreatic beta cells [36].

During physical activity, the release of oxygen to the active muscle increases, which can result in elevated levels of free radicals. These radicals are generated in significant quantities within cells where electron transfer reactions occur. The electron then separates from the electron transport cycle and reacts with oxygen, thereby increasing antioxidants such as SOD and GPx in order to reduce or eliminate free radical activity [37].

Additionally, the results of the present study demonstrated that the groups receiving the Sup and the combination of Sup and AT exhibited diminished levels of MDA in comparison to the Con group. Qaraqat is a rich source of antioxidants, which prevent an

increase in lipid oxidation and lead to an increase in antioxidant activity. This is due to the presence of flavonoids, which reduce lipid peroxidation and, in turn, reduce MDA levels [38]. The decrease in MDA, which is an indicator of oxidative stress and is produced in response to consumption, can be attributed to the presence of polyphenolic compounds and the antioxidant activity of Qaraqat [38]. It may therefore be beneficial to consume Qaraqat as a means of preventing the development of atherosclerosis. This is achieved by inhibiting LDL oxidation and preventing oxidative stress [38].

Con group exhibited lower levels of antioxidant enzymes SOD, TAC, and GPx in comparison to AT group. These findings suggest that aerobic training may enhance antioxidant capacity. This finding was consistent with the results of Klarod et al. (2023) which demonstrated that eight weeks of exercise had a positive impact on antioxidant balance [16]. The results of this study were also in accordance with the findings of Akbarpour et al. (2023) indicating that eight weeks of traditional resistance training and TRX increased SOD and GPX levels and decreased MDA [18].

The adaptation of exercise training has been demonstrated to enhance antioxidant activity and mitigate oxidative damage [39]. In light of the beneficial impact of physical activity on SOD, GPx may potentially elucidate several underlying mechanisms. The first mechanism is Nrf-2 in which physical activity increases its phosphorylation [40]. It can be concluded that the exercise-induced activation of Nrf-2 provides an antioxidant protective mechanism by binding to the antioxidant element response, which is involved in the promotion of several antioxidant enzymes, including MnSOD [40]. Also, exercise leads to an increase in SIRT1, which in turn increases the activity of the antioxidant system and improves the cellular DNA repair cycle [41].

Another mechanism by which oxidative stress induced by endurance training may induce PGC1-α protein expression is through the decrease in endogenous antioxidant glutathione that occurs along with endurance activity. PGC1-α can lead to the transcription of CREB combined protein factor, which in turn increase free radical detoxification enzymes such as GPx and MnOD. To activate these antioxidants, PGC1-α combines with ERR-α and induces mitochondrial activation in the matrix [41].

Additionally, the results of the present study demonstrated that FBG and insulin resistance levels in the Sup group were significantly lower than those observed in Con group. The plant in question contains a substantial quantity of anthocyanins, which are bioflavonoid complexes present in Qaraqat. These anthocyanins possess a range of beneficial properties, including anti-inflammatory, anti-cancer, and antioxidant effects [21]. Qaraqat is a type of herbal insulin that decreases blood glucose by inhibiting the uptake of glucose from the intestines, inhibiting gluconeogenesis in the liver, and increasing the uptake of glucose by cells [42]. The gluconic compounds present in Qaraqat have insulin-like properties, while the leaves of this plant contain chromium, which is effective in controlling blood glucose [43].

The anthocyanins present in Qaraqat inhibit the absorption of glucose from the intestines. This is achieved by inhibiting the enzyme alpha-amylase and activating the (GLUT4) in muscles and fat. This result in

increased glucose uptake and ultimately leads to a decrease in blood glucose levels [44]. Anthocyanins also reduce gluconeogenesis in the liver by inhibiting the hepatic glucose-6 phosphatase enzyme [45]. The reduction of insulin resistance is achieved by the decrease in the expression of TNF-α, MCP-1, and IL-6 in adipose tissue, which is brought about by the action of anthocyanins. Furthermore, anthocyanins have been demonstrated to reduce blood glucose levels by increasing the expression of AMPK, which in turn increases the expression of GLUT4 in muscles and adipose tissues while simultaneously decreasing protein-binding retinol 4.

Also, Qaraqat supplementation lowers blood glucose by activating the active protein kinase AMP [21]. Idris and Al-Ubaidi (2010) [46], and Mani et al. (2012) [47] demonstrated that the consumption of Qaraqat supplements can significantly reduce FBG levels. Also, Barut et al. (2019) concluded that Qaraqat has antioxidant effects [22]. This finding was in alignment with the results of the present study.

Qaraqat is a plant with documented antioxidant properties, which may confer healing benefits [38]. Antioxidants are defined as molecules that can neutralize free radicals before they attack cells, thereby preventing the breakdown of the body's natural defense. Antioxidants may reduce the level of free radicals by two principal mechanisms: first, by the ability or expression of free radical enzymes such as NADPH oxidase and xanthine oxidase; secondly, by increasing the activity and expression of antioxidant enzymes such as SOD or GPX [48]. Also, antioxidants can reduce their reactivity by donating electrons to free radicals. Moreover, the deactivation of the initiator calcium can lead to the removal of active agents or initiators. Active species of nitrogen [49].

5. Conclusions

According to the results of the present study, eight weeks of aerobic training together with Qaraqat supplementation and separately can have beneficial effects on diabetes and antioxidant factors. Therefore, it can be concluded that the consumption of Qaraqat supplement in diabetic conditions can be as beneficial as physical activity, and it can be applied together with aerobic training to optimize the effect of Qaraqat supplement. In future research, researchers can add more information to this field of research by considering more blood factors to investigate the effects of Qaraqat supplements separately and in combination with various types of aerobic and non-aerobic training.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The authors have completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. This study was approved by the Ethics Committee of Tabriz University (Ethics Code: IR. TABRIZU. REC.1402.037)..

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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