The effect of progressive endurance training and extract of black winter truffle on proteins levels and expression of hippocampus α-synuclein and HSF1 in the healthy and diabetic rats

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ARTICLE INFO

ABSTRACT

Aim: The research aimed to investigate the effect of endurance running and *T. Brumale* extract on α-Syn and HSF1 in the brain and serum of healthy and diabetic rats.
Methods: A total of 40 Wistar rats were randomly divided into eight groups: Control (C), Exercise (E), Control-Tuber (T), Exercise-Tuber (ET), Control-Diabetes (D), Exercise-Diabetes (ED), Control-Diabetes-Tuber (CDT), and Exercise-Diabetes-Tuber (EDT). The endurance running was carried out five times per week for five weeks. The hippocampus and the serum α-Syn and HSF1 were measured using an enzyme-linked immunosorbent assay method.
Results: The brain α-Syn levels were higher in diabetic groups than in the healthy groups, but insignificantly ($P \leq 0.05$). The brain α-Syn level significantly increased in the EDT group compared to the T group ($P \leq 0.05$). The serum level of α-Syn in the ED group was significantly higher than in the E and D groups ($P \leq 0.05$). The brain HSF1 level was significantly higher in the ED group compared to the D group ($P \leq 0.05$). The gene expression of *hsf1* was significantly reduced in the E group compared to the other groups and the EDT group compared to ED and CDT groups ($P \leq 0.05$). Furthermore, the serum HSF1 level significantly increased in the ED group compared to the D group ($P \leq 0.05$).
Conclusion: The results of this study suggest that progressive endurance running may improve neuroprotective conditions in diabetic patients by increasing HSF1 in the brain.

1. Introduction

Diabetes mellitus is a disorder resulting from defective insulin secretion, resistance to insulin action, or both, which can cause a disturbance in the performance and structure of many body tissues, including; the heart, kidneys, eyes, skin, and especially the brain [1]. Diabetes is known as one of the Neurodegenerative factors in the brain, especially in the hippocampus and cortical regions, and is increasingly associated with an increased risk of developing Parkinson's disease (PD) [2].

Alpha-synuclein (α-Syn) is a cytosolic neuronal protein, and its aggregation is considered a major pathogenic event in PD. Some studies have indicated that the oligomers formed during the α-Syn aggregation process are the strongest neurotoxic species involved in the pathogenesis of PD [3].

A massive bulk of studies have shown that the expression and accumulation of α-Syn increases in various tissues of diabetic subjects, including; the cerebellum [4], substantia nigra of the midbrain, pancreas [5] and cerebrospinal fluid (CSF) [6]. Recent data demonstrated the close and potential association between diabetes and neurodegenerative diseases (Parkinson's and Alzheimer's diseases) which indicates that diabetes may facilitate the onset, development, and progression of these diseases [2,7]. On the contrary, some studies have revealed that *α-syn* expression decreased in the hippocampus and cerebellum of diabetic rats, but this decrease was not significant [8]. In this regard, the results of another study on animals affected by middle cerebral artery occlusion showed that *α-syn* expression was more widely reduced in the cerebral cortex of diabetic subjects compared to non-diabetic subjects. These findings suggest that a reduction of *α-syn* expression causes more extensive brain damage in ischemic injury with diabetes [9]. However,

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Leino et al. (2017) reported that the incidence and extent of human brain α-Syn increased with age, while no major differences were observed between diabetic and non-diabetic subjects [10].

Heat shock factor 1 (HSF1) is an essential stress-responsive transcription factor that plays a pivotal role in the proteotoxic stress response which protects cells against protein misfolding, aggregation, and apoptosis [11]. HSF1 has been suggested as potentially therapeutic for neurodegenerative diseases such as; Alzheimer's, Parkinson's, and Huntington's. It is well demonstrated that increasing or activating HSF1 has potent neuroprotective effects [12]. Some studies have investigated the association between α-Syn and HSF1. For instance, Liangliang et al. (2010) reported that the expression of *hsf1* dramatically reduced the levels and cytotoxicity of α-Syn and significantly protects SH-SY5Y cells from α-Syn-induced toxicity [13]. In contrast, another study revealed aberrant HSF1 degradation by aggregated α-Syn in SH-SY5Y cells [14].

Numerous studies have shown that exercise prevents the increase of α-Syn in the brain of Parkinson's patients [15] and can significantly reduce the levels of this protein in the brain [16] and red blood cells [17] of patients. However, Zhou et al. (2017) revealed that 3 months of voluntary exercise on a running wheel reduced the levels of α-Syn transgenic mice' brains and increased its levels in plasma [18]. Literature review shows that few studies are carried out on the effect of exercise on HSF1; some studies have reported that acute exercise can activate HSF1 in the myocardium [19]. However, some studies showed that an aerobic exercise bout [20] and two days of treadmill running at 70% VO$_2$max [21] does not exert an effect on skeletal muscle and cardiac HSF1 levels, respectively.

Recently, bioactive constituents of numerous plant extracts have been examined in neurodegenerative diseases. Research findings have shown that phytochemical compounds in plants, including polyphenols, alkaloids, terpenoids, and glucosides, are effective in different stages of α-Syn pathogenesis (aggregation, fibrillation, elongation, nitration, and oligomerization) and reduce the protein levels in experimental models [22]. Besides, it is also reported that some plant compounds, including phenols, polyphenols, glucosides, and terpenoids; are able to induce, express, and activate HSF1 [23].

Truffles, a type of underground edible fungus, are rich in unique and essential bioactive compounds which are related to their antioxidant, anti-inflammatory, antidiabetic, antitumor, hepatoprotective, and immunomodulatory properties. *Tuber brumale*, also known as winter truffle, is one of the most valuable gastronomic truffle species worldwide. In this regard, only a study showed that the low dose of tropical tuber extract (*Dioscorea alata* L.), through regulating HSF1 and SKN-1/Nrf2 signaling pathways, reduced α-Syn accumulation [24].

Given the above evidence that the levels of α-Syn and HSF1 proteins are effective agents in neurodegeneration and neuroprotection, they are inversely activated in neurodegenerative diseases; Therefore, the current research is intended to investigate the effect of endurance exercise and *T. brumale* extract on the expression of *α-syn* and *hsf1* proteins in the hippocampus of diabetic rats.

2. Methods

2.1. Animals

Male Wistar rats ($n = 40$ and weight range of 200–250 g) were purchased from the animals' center of Pars institute of Iran. Animals were housed in the exercise physiology faculty, at the University of Mazandaran (12/12 h light/dark cycle at 22 ± 2 °C). Animals were kept in groups of three in polycarbonate cages, which have free access to water and standard rat food. After one week of acclimatization period to the new environment, three rats were selected as the pilot diabetic group. After a pilot study, animals were randomly divided into eight groups: 1. Control (C), 2. Exercise (E), 3. Control-Tuber (T), 4. Exercise-Tuber (ET) 5. Control-Diabetes (D), 6. Exercise-Diabetes (ED), 7. Control-Diabetes-Tuber (CDT), and 8. Exercise-Diabetes-Tuber (EDT).

2.2. Induced diabetes

Diabetes was induced after 12 h of food deprivation by IP injection of streptozotocin (STZ) solution (Zellbio, Germany), 50 mg/kg dissolved in 0.5 mol/L of citrate buffer (pH:4.5, 0.1 M). After 48 h, blood samples were taken from the tails of rats, and fasting blood glucose concentration was measured by ACCU-CHEC glucometer. Rats with blood glucose levels were higher than 300 mg/dl, as diabetic [25].

2.3. Exercise training

After STZ-induced diabetes and familiarization week to treadmill exercise (10–15 min, speed 10 m/min, 5 days/week), all training groups completed five weeks (5 sessions per week) of study. The intensity and volume of training sessions increased progressively over the intervention period [25]. Furthermore, 3 min were allocated to warm up and cool down. Rats received up to 10 shocks of 0.5-s duration over the last two weeks of the training period (Table 1).

2.4. Tuber brumale extract preparation

Tuber brumale, called the black winter truffle, was purchased from Mazandaran province, north of Iran, cleaned, washed with tap water, and dried for one day in a lab oven at 40 °C. Hydroalcoholic extract of black truffle was prepared according to Zhang et al., 2018 [26]. Four groups (CT, ET, CDT, and EDT) received 400 mg/kg BW of *T. brumale* extract at the end of each training session for 5 weeks. To equality, these conditions, normal saline was given to the other groups orally (gavage) in equal volumes.

2.5. Identification of GC-MS

The Agilent technologies 7890 A gas chromatograph (GC) device connected to the Agilent 5975C mass spectrometer consisting of an HP-5MS 30 m × 0.25 mm, 0.25 μm column was used. Column temperature was set at 100 °C with 10 °C per minute increment. At 200 °C, the temperature was kept constant for 5 min and then increased to 280 °C at 3 °C per minute and kept at this temperature for 5 min. Also, the GC chromatogram obtained from the analysis of volatile components obtained from *T. brumale* extract is shown in Fig. 1.

2.5.1. Chemical compounds in the iranian truffle extract based on GC-MS

Identification of the constituents of *T. brumale* extract was performed using MS-GC device and the results are shown in Table 2.

2.6. Serum collection and tissue biopsies

Rats were anesthetized by injection of xylazine (3–5 mg/kg body mass) and ketamine (30–50 mg/kg body mass), 24 h after the last training session. Twelve hours before sacrifice, food (not water) was removed. Blood samples were collected from the inferior vena cava next to the heart. The hippocampus tissues were excised, cleaned, washed with normal saline and frozen in liquid nitrogen, and stored at −80 °C for further analysis.

Table 1
Exercise protocol.

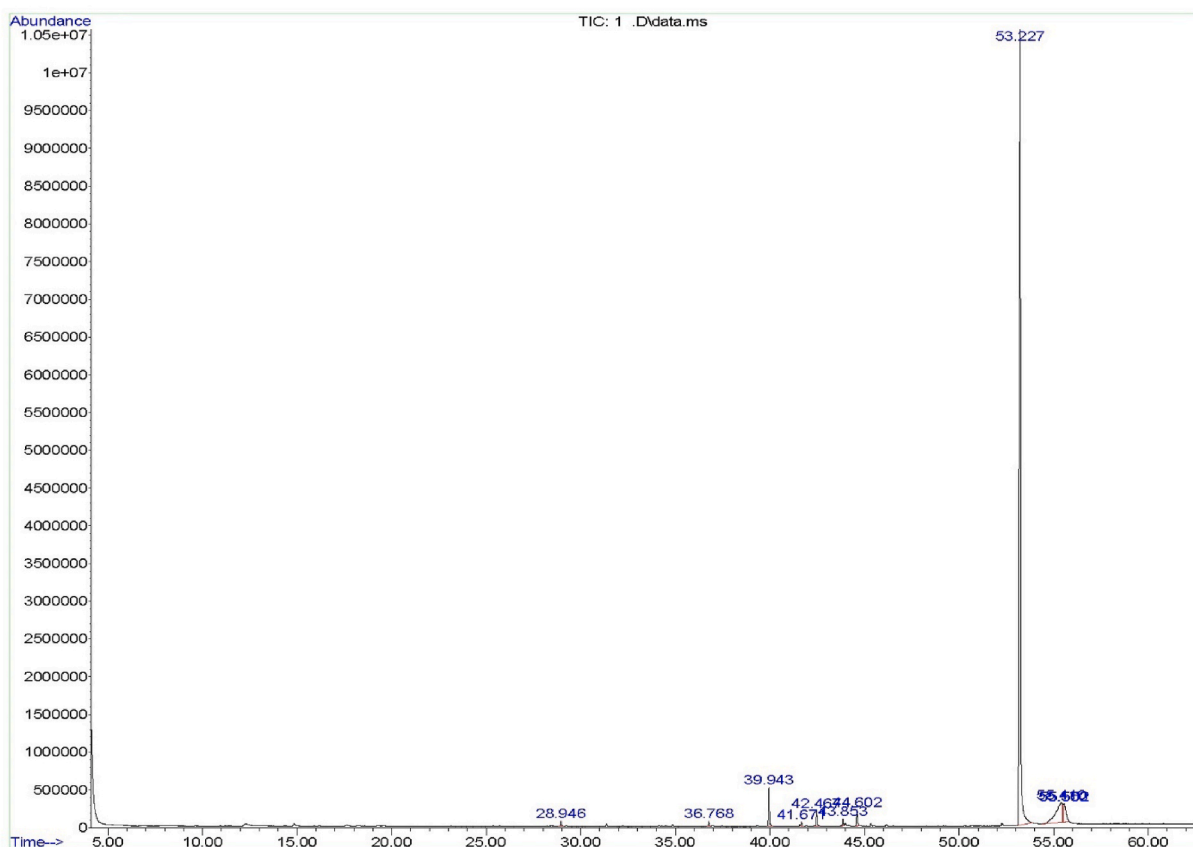| Weeks (Familiarization/Training)	Duration	Velocity
F	10–15 min	10 m/min
T (1)	10 min	10 m/min
T (2)	20 min	10 m/min
T (3)	20 min	15 m/min
T (4)	30 min	15 m/min
T (5)	30 min	18 m/min

Fig. 1. GC-MS chromatogram from analysis of components of *T. brumale* extract.

Table 2

Chemical compounds of T. brumale extract.

Chemical compound	Time	Relative abundance (%)
Phenol, 2,4-bis(1,1-dimethylethyl)	28.94	0.34
Octadecane	36.77	0.37
Hexadecanoic acid, methyl ester	39.94	2.68
Eicosane	41.68	0.28
Methyl 4-methylpentan	42.46	1.11
9,12-Octadecadienoic acid	43.85	1.48
Methyl stearate	44.6	1.68
-4-Benzenesulfonyl-3,5,6 Trifluoro	55.04	13.68
bicyclo 5.3 0 decan 2 one 9- (diphenylmethylene)-	55.50	0.70

2.7. Statistical analysis

All data are presented as mean $\pm$ SEM (standard error of the mean). One-way ANOVA was used to detect differences among groups with the GraphPad Prism 8.4.3. The normality of this data was assessed using the Shapiro-Wilk test. As a reference for Tukey's multiple comparisons post hoc test, α-Syn and HSF1 data were used. $P \leq 0.05$ was considered the significant statistical level.

3. Results

3.1. Effects of exercise and tuber extract on hippocampal α-Syn protein and its gene expression

Based on the results obtained, it was revealed that the hippocampal α-Syn levels were higher in the diabetic groups than in the control groups, though insignificantly. Our results showed that there was a tendency to increase in the sedentary group consuming (CDT) extract compared to other diabetics (D, ED, and EDT) groups. Nevertheless, the level of α-Syn protein in sedentary diabetic rats consuming the extract (CDT group) was significantly increased compared to sedentary healthy rats consuming the extract (T group), (Fig. 2).

Additionally, results of the present study revealed that the gene expression of *α-syn* hippocampus increased in the E group and decreased in the ET group compared to the control group in healthy rats; however, these alterations were not statistically significant. There was also an insignificant decrease in *α-syn* gene expression in the D group compared to the C group (Fig. 3).
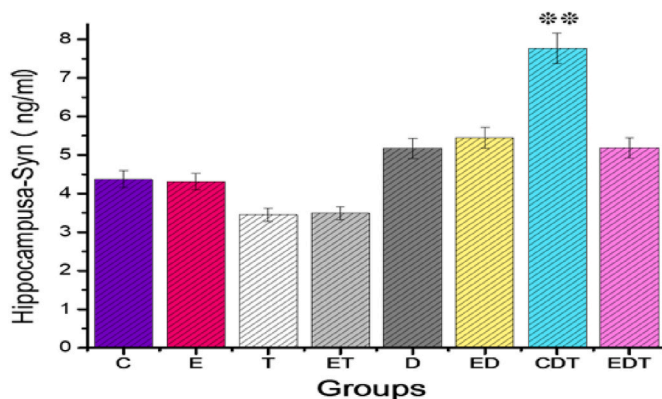

Fig. 2. Comparison of hippocampal protein level of α-Syn in the controlled and treated groups at the end of the study period. **: $P < 0.05$ compared to the T group.
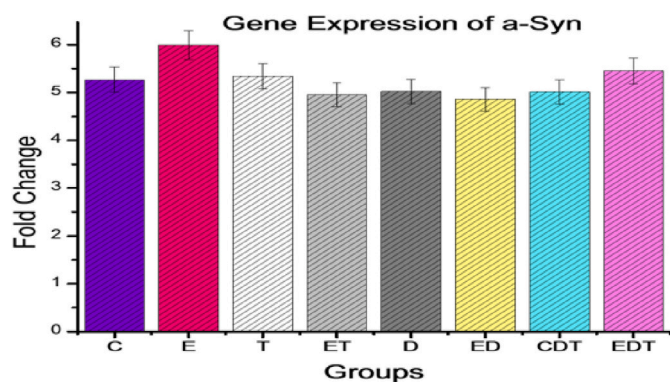
Fig. 3. Comparison of hippocampal expression of *α-syn* in the controlled and treated groups at the end of the study period.

3.2. Effects of exercise and tuber extract on serum α-Syn

Serum levels of α-Syn tended to increase in the E group compared to the control group, while serum levels of this protein tended to decrease in the T and ET groups compared to the C and E groups. Also, a statistically insignificant increase in serum α-Syn levels was observed in the diabetic group (D) compared to the control group (C). However, our results revealed a significant increase in serum α-Syn levels in the ED group compared to the E group. Research findings also revealed that exercise (ED group) caused a significant increase in serum α-Syn levels compared to the control (D) group in diabetic rats. However, the interactive effect of exercise and extract consumption (EDT group) significantly reduced serum levels of this protein compared to control (D) and exercise (ED) groups in diabetic rats (Fig. 4).

3.3. Effects of exercise and tuber extract on hippocampal HSF1 protein and its gene expression

In this study, the effects of exercise, tuber extract, and diabetes on hippocampal HSF1 were investigated. The results showed that exercise (E group) increased HSF1 compared to the control group (C) of healthy subjects. Conversely, extract consumption in T and ET groups showed a decrease in HSF1 hippocampal protein levels; however, these changes were not significant. Although the levels of this protein in the brain decreased in the D group compared to the C group, the alterations were not significant. However, exercise significantly increased HSF1 protein levels in ED group compared to the D group. The results also revealed that exercise and consumption of tuber extract simultaneously could
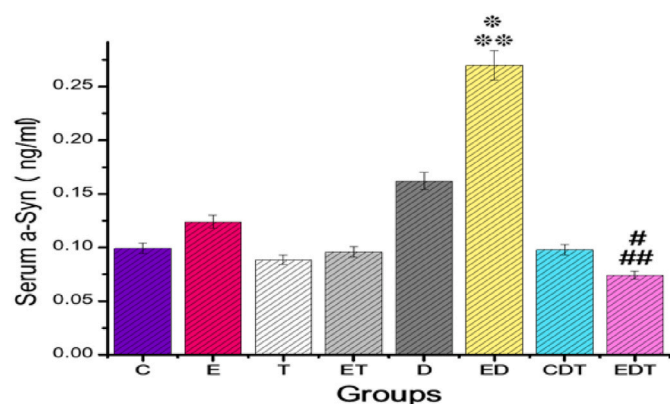
insignificantly increase HSF protein levels in the EDT group compared to the D group (Fig. 5).

Our study showed that hippocampal expression of *hsf1* significantly reduced in the E group compared to the C group. The results also showed that the gene expression of *hsf1* did not significantly change in the T and ET groups compared to the C. However, a comparison of the results of the E group with T, ET, and ED groups showed that the expression of this gene in the E group could be significantly reduced. The results also showed that the use of tuber extract in the T group and exercise and consumption of tuber extract simultaneously in the ET group could insignificantly decrease its expression. Also, it was found that the gene expression of the *hsf1* did not significant change in the D group compared to the C group at the end of the period. However, a comparison of the results of the diabetic groups showed that the diabetic group, which underwent exercise and received tuber extract at the same time (EDT group), could significantly reduce the expression of this gene compared to the D, ED, and CDT groups (Fig. 6).

3.4. Effects of exercise and tuber extract on serum HSF1

In the current study, too, the level of HSF1 in serum was measured to evaluate the effect of exercise and tuber extract consumption in diabetic and healthy animals. The results of statistical analysis of these data showed that the serum level of HSF1 insignificantly increased in the T group compared to the C, E, and ET groups. Although the serum level of HSF1 in the E and ET groups were lower than in the control group, the alterations were not significant. The findings of the present study showed that the serum level of HSF1 increased in the diabetic group (D) compared to the control group (C), but this increase was not significant. Serum levels of this protein were also significantly higher in diabetic animals which performed endurance running five sessions a week for five weeks (the ED group) than in diabetic animals which did not receive any intervention (the D group). Additionally, a comparison of findings among diabetic groups showed that exercise and consumption of tuber extract in diabetic animals (the EDT group) could significantly reduce the serum level of the protein compared to the ED group. However, a significant difference between the EDT groups with the D group was not reported. Although serum HSF1 levels were higher in the diabetic rats consuming tuber extract (CDT group) than in the D group, a significant difference was not observed between the two groups (Fig. 7).

4. Discussion

To date, no study has been found on the effect of exercise and consumption of *T. brumale* extract on α-Syn levels in the brain and blood of diabetics.

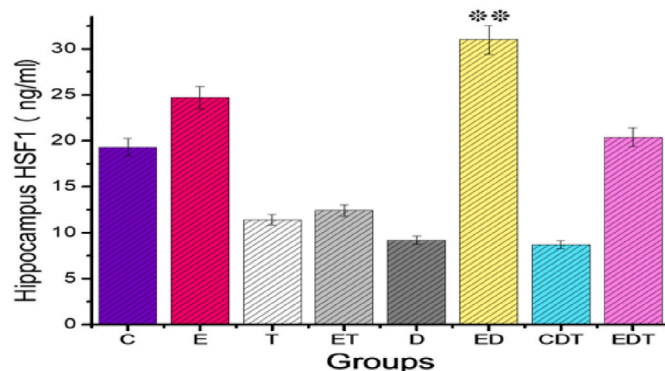The results of our study showed that 5 weeks of diabetes increased

Fig. 4. Comparison of serum level of α-Syn in the controlled and treated groups at the end of the study period. *: P < 0.05 compared to the D group, **: P < 0.05 compared to the E group, #: P < 0.05 compared to the D group, ##: P < 0.05 compared to the ED group.

Fig. 5. Comparison of hippocampal protein level of HSF1 in the controlled and treated groups at the end of the study period. **: P < 0.05 compared to the D group.
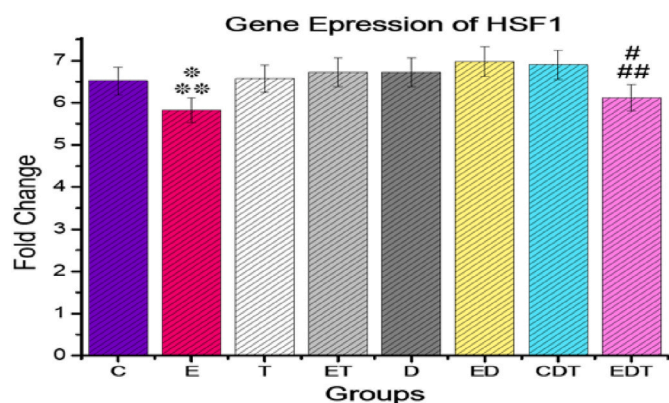
Fig. 6. Comparison of hippocampal expression of *hsf1* in the controlled and treated groups at the end of the study period. *$P < 0.05$ compared to the C group, **$P < 0.05$ compared to the T, ET and ED groups, #$P < 0.05$ compared to the D group, ##$P < 0.05$ compared to the ED and CDT groups.
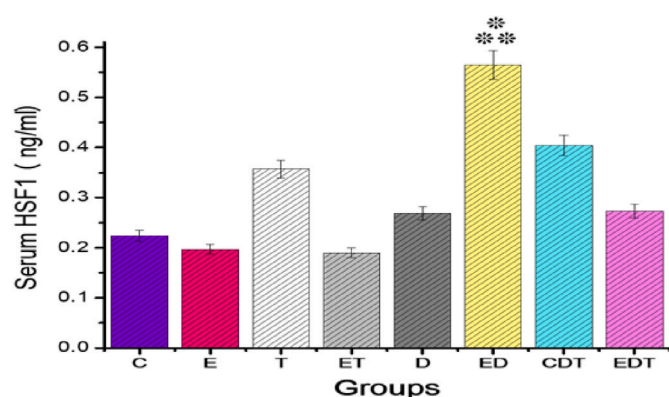

Fig. 7. Comparison of serum level of HSF1 in the controlled and treated groups at the end of the study period. *$P < 0.05$ compared to the D and EDT groups, **$P < 0.05$ compared to the E group.

the accumulation of oligomeric α-Syn protein in the brains of diabetic groups, but this increase was not significant. These results are not in line with recent results [4,7,27]. In these studies, in which subjects had experienced diabetes for at least 7 weeks, only significant α-Syn monomeric accumulation was observed [4,7,27], and only one study in db/db and ob/ob mice aged 2.5 months showed significant both monomeric and oligomeric accumulation of α-Syn [5]. Studies have shown that the imbalance between the production and clearance of α-Syn causes the accumulation of α-Syn monomer and its misfolding and incorrect conversion into its toxic form, oligomer [28]. The formation of oligomers indicates toxicity of α-Syn function and plays a very important role in neurodegeneration and possibly the collapse of neural homeostasis [29]. Therefore, it seems that observing significant changes in toxic α-Syn oligomer accumulation in the brain will require more time and progression of the disease. Increased accumulation of α-Syn in brain diabetic subjects may be caused by impaired insulin signaling, increased oxidative stress and inflammation markers [4,5], decreased interaction of HSPB5 (αBC) with α-Syn and p-Tau due to hyperglycemia [27], and colocalized with amylin [7].

The results of the present study showed that oligomeric *α-syn* expression in diabetic brain tended to decrease compared to healthy individuals, however, no significant difference was observed. In this regard, various studies have shown conflicting results, including a decrease [8,9], increase [4,5], and no change [10]. The reason for the difference in these results is not clear. However, it seems that part of it may be due to the lack of complete knowledge of the mechanisms

involved in this process and others may be related to the different protocols used in similar studies, the duration, severity of disease, and the age or sex [4,5,8–10].

The results of the present study showed that the serum α-Syn levels increased in the diabetic rats compared to healthy ones; however, it was not significant. These results contradict the findings of Rodriguez et al. (2015) who reported that serum α-Syn levels decrease in an age-dependent behavior in db/db mice. They showed that low blood α-Syn level is associated with increased serum insulin levels as well as insulin resistance in diabetic patients [30]. In the present study, type 1 diabetes was induced, as a result of which insulin-producing beta cells are destroyed, and also this type of diabetes is not associated with insulin resistance [31]. Therefore, other factors besides insulin appear to play a role in serum α-Syn changes. On the other hand, local and systemic inflammation caused by diabetes can induce breakdown and increase the permeability of the brain-blood barrier (BBB) and also reduce the removal of waste and increase the infiltration of immune cells [32]. Following this inflammatory state, the BBB allows extracellular vesicles derived from red blood cells to transport α-Syn (possibly in oligomeric form) from the peripheral circulation to the brain [33]. Accordingly, it appears that diabetes may increase brain α-Syn levels by increasing α-Syn transfer from the blood to the brain, which may play a role in the onset and development of neurodegenerative diseases. In line with this propose the results of other research on Parkinson's patients have shown higher oligomeric α-Syn concentrations in the blood [34] and peripheral tissues [35].

The present study showed that exercise did not significantly alter the accumulation and expression of hippocampal α-Syn in diabetic subjects. However, serum oligomeric α-Syn levels in active diabetics increased significantly compared to healthy active and inactive diabetic rats, which is in line with the findings of Zhou et al. (2017) who showed 3 months of voluntary running decreased oligomeric α-Syn in the brain and increased monomeric, dimeric and total α-Syn in plasma of Parkinson's mice [18]. Given the 5-week duration of our study, exercise appears to prevent a significant increase in the brain α-Syn of diabetic rats by increasing the α-Syn clearance from the brain to the bloodstream. Moreover, some studies indicated that α-Syn increased in peripheral tissues in some pathological conditions. Both long-term endurance exercise or high-intensity exercise and persistent hyperglycemia/diabetes can cause oxidative stress in blood and skeletal muscle [36]. Oxidative stress strongly contributes to abnormal protein misfolding and the production of oligomers and is also associated with the accumulation of α-Syn misfolded proteins [17]. As a result, it seems that the increase in oxidative stress caused by diabetes and the subsequent increase in oxidative stress by exercise can be considered as a possible mechanism to increase the accumulation of α-Syn oligomers in the blood of active diabetic rats.

Our study is the first one on the effect of *T. brumale* extract consumption on α-Syn protein levels in diabetics. It showed that brain α-Syn was significantly increased and there was a tendency to decrease this protein in the serum of diabetic animals consuming tuber extract. However, some studies have shown polyphenolic compounds [37] and extracts of some plants [38] reduced the expression and accumulation of α-Syn in the brain. Considering the reducing effect of tuber extract on the α-Syn brain of healthy subjects in our study, it appears that diabetes is an important factor in stimulating the increase of this protein despite the consumption of the extract. It seems that the consumption of the extract improved the function of the BBB and did not allow the α-Syn oligomer, which was increased due to the induction of diabetes, to completely escape from the brain to the blood. As a result, despite the consumption of the extract, it causes a significant increase in α-Syn in the brain of diabetic rats. While studies show that free α-Syn can cross the BBB in both directions from blood to brain and from the brain to blood in healthy and inflammatory conditions [33]. However, the understanding of its mechanism is unclear and the discovery of its possible mechanism requires further investigation in the future. Nevertheless,

the serum levels of α-Syn in active diabetic subjects who received tuber extract showed a significant decrease compared to active and sedentary diabetic subjects (control group) who did not consume *T. brumale* extract. It seems that consumption of tuber extract and exercise activity by reducing blood glucose levels [26], regulating Nrf2 and NF-kB pathways, and regulating enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, and vitamins E and C [26] as well as the protective effect on pancreatic β-cells in diabetics [39] may be involved in decreasing serum α-Syn levels. Due to the significant decrease in serum α-Syn level of active and sedentary diabetics consuming the tuber extract compared to the diabetic control group, it is suggested that consumption of tuber extract alone, when compared to exercise alone, had a better effect on reducing serum α-Syn.

Our results showed that hippocampus HSF1 protein levels decreased in the diabetic rats compared to healthy group; however, it had no significant. Consistent with our results, Reddy et al. (2021) reported that HSF1 protein levels were significantly reduced in the cerebral cortex of diabetic subjects 16 weeks after the onset of diabetes [27]. In addition, contrary to Reddy's results, no change in the expression of *hsf1* was observed in diabetic subjects compared to healthy subjects, which could be due to the difference in the duration of the study period and the severity of the disease [27]. Moreover, our results showed no change in serum HSF1 levels of diabetic rats compared to healthy ones. Some studies have reported conflicting results for the expression of HSF1 expression and protein level in the peripheral tissues of diabetic patients, including decrease [40,41], increase [42], and no change [43]. Given the differences and contradictions in the production of HSF1 in different peripheral tissues of diabetics, as well as the results of our research, the discovery of the relationship between HSF1 levels in the bloodstream and peripheral tissues is unclear and at the same time ambiguous. Hence, more research seems to be needed.

Despite the tendency to increase HSF1 levels in the brain of active healthy subjects compared to sedentary healthy subjects, we observed exercise significantly reduced the serum levels and brain expression of HSF1. Studies have shown that (acute and chronic) exercise or physical activity can increase protein level and expression of HSF1 in the liver [40], heart [19,40,44] and skeletal muscle [40,45]. However, other studies showed that two consecutive days of running on a treadmill with 70% VO$_2$max and one cycling session with 60% VO$_2$peak had no effect on HSF1 levels in the heart [21] and skeletal muscle [20] of healthy subjects, respectively. It is well documented that exercise improves the body's antioxidant system and prevents oxidative stress in peripheral and central tissues, especially the brain [46]. Furthermore, HSF1 is an important factor related to the antioxidant system and oxidative stress can activate HSF1 independently and dependently on heat shock. Therefore, it appears that the significant decrease in brain *hsf1* gene expression and serum HSF1 level of active healthy individuals observed in our study could be associated with a reduction in oxidative stress and an increase in antioxidant capacity due to regular exercise [47].

Findings from our study showed that exercise significantly increased the accumulation of HSF1 protein levels in the hippocampus and serum of active diabetics compared to sedentary diabetics, and also tended to increase its expression in the brain of these subjects. In this regard, Atalay et al. (2004) showed that endurance training increased *hsf1* activity and expression in the peripheral tissues of diabetic animals such as the heart, skeletal muscle, and liver [40]. It seems that progressive exercise by increasing some factors such as temperature and ROS, which activate the intracellular signaling pathway of adrenergic stress, leads to an increase in the heat shock response induced by HSF1. Thus, exercise may increase HSF1 through mechanisms such as increasing HSP70 and BDNF [19] and inhibiting GSK3 in the brain. Furthermore, some studies reported that aerobic exercise upregulates AMPK and SIRT1 [48]. Hence, it seems to explain the increase in the brain HSF1 levels caused by the endurance exercise performed in our study.

The present study showed that consumption of tuber extract alone reduced the accumulation of HSF1 in the hippocampus and also

increased serum levels of this protein in healthy and diabetic groups, but not significantly. In addition, *hsf1* expression tended to increase in diabetic subjects. It is well known that the tuber is rich in antioxidants and anti-inflammatory and in some studies, its anti-diabetic properties have been proven [49]. Consistent with our study, Jiang et al., reported that *Tuber melanosporum*, another subspecies of tuber, contains the antidiabetic and antinephritic properties of tuber that could mediate by modulating oxidative stress and inflammation-related cytokines through improving the Nrf2 signaling pathway [49]. Moreover, in line with our evidence, Pan et al. (2021) indicated that consumption of a mushroom called *Ganoderma lucidum* polysaccharide could increase *hsf1* expression in the hepatocytes of T2DM mice [50]. However, another study showed that quercetin significantly reduced *hsf1* expression in the pancreas tissue of diabetic rats [42]. It appears that cellular pathways other than oxidative pathways are involved in this response. Some studies also indicated that glucolipotoxicity induced by diabetes could inhibit HSF1 activity via acetylation [41]. It appears that truffles that have antioxidant, anti-inflammatory, and anti-diabetic properties [24] might inhibit glucolipotoxicity and reduce its negative effects by improving glucose and lipid metabolisms. Hence, it is probably one of the mechanisms tending to increase the expression of this protein in diabetic animals.

Several studies have also shown an inverse relationship between α-Syn and HSF1 [13,14]. Some of these studies revealed that the abnormal accumulation of α-syn correlated with the progression of neurodegenerative diseases. While HSF1 provides neuroprotection against α-synucleinopathy [14]. Nevertheless, our results showed that although diabetes caused a tendency to increase α-Syn and decrease HSF1, these changes were not significant. Therefore, no clear conclusion can be drawn from the findings of the present study. In addition, our results showed that exercise significantly increased HSF1 but did not change α-Syn levels in the brain. Also, consumption of extract did not change HSF1 but increased α-Syn levels in the brain. At the same time, the combination of exercise and extract did not change the levels of the two proteins, but it significantly decreased the expression of *hsf1*. Our study showed that disease induction could show this inverse relationship to some extent, but exercise alone and extract consumption alone or in combination cannot show such an inverse effect. It seems that the possible inverse relationship between these two proteins, which was reported in some other neurodegenerative diseases such as Parkinson's, is changed by the induction of diabetes in a different way and through other mechanisms. However, future studies should focus on a longer duration of diabetes and with/without exercise and extract therapy to determine the complication of the disease.

In summary, it can be concluded that during 5 weeks, only physical activity may improve the neuroprotective status of the diabetics' brain by increasing HSF1. Also, the interaction of physical activity and herbal extract consumption may reverse the neurodegenerative condition. Moreover, it appears that more time is needed to observe the effect of tuber plant extract on diabetic people.

Although we encountered some limitations in the present study, we can suggest some of them to be carried out in future studies, including conducting an immunohistochemistry study to investigate the distribution and accumulation of proteins and the induction of type 2 diabetes model using a high-fat diet and streptozotocin for a Longer period of disease.

Funding

CRediT authorship contribution statement

administration, Funding acquisition, Supervision, Methodology, Writing – original draft, Writing – review & editing. **Abolfazl Akbari:** Writing – review & editing, Validation.

References

[1] Zhao Y, et al. Prevalence of other diabetes-associated complications and comorbidities and its impact on health care charges among patients with diabetic neuropathy. J Diabet Complicat 2010;24(1):9–19.

[2] Hong CT, et al. Insulin resistance promotes Parkinson's disease through aberrant expression of α-synuclein, mitochondrial dysfunction, and deregulation of the polo-like kinase 2 signaling. Cells 2020;9(3).

[3] Mehra S, Sahay S, Maji SK. α-Synuclein misfolding and aggregation: implications in Parkinson's disease pathogenesis. Biochim Biophys Acta, Proteins Proteomics 2019;1867(10):890–908.

[4] Solmaz V, et al. Accumulation of α-synuclein in cerebellar purkinje cells of diabetic rats and its potential relationship with inflammation and oxidative stress markers. Neurol. Res. Int. 2017;2017:5952149.

[5] Wang L, et al. Metabolic inflammation exacerbates dopaminergic neuronal degeneration in response to acute MPTP challenge in type 2 diabetes mice. Exp Neurol 2014;251:22–9.

[6] Pagano G, et al. Diabetes mellitus and Parkinson disease. Neurology 2018;90(19):e1654–62.

[7] Sun Y, et al. Cynomolgus monkeys with spontaneous type-2-diabetes-mellitus-like pathology develop alpha-synuclein alterations reminiscent of prodromal Parkinson's disease and related diseases. Front Neurosci 2020;14:63.

[8] Liu Y, He R. Fasting induces a high level of 3-nitrotyrosine in the brain of rats. Neurosci Lett 2010;472(3):204–9.

[9] Kang JB, et al. Hyperglycemia aggravates decrease in alpha-synuclein expression in a middle cerebral artery occlusion model. Lab. Anim. Res. 2018;34(4):195–202.

[10] Leino M, Popova SN, Alafuzoff I. Transactive DNA binding protein 43 rather than other misfolded proteins in the brain is associated with islet amyloid polypeptide in pancreas in aged subjects with diabetes mellitus. J. Alzheim Dis. 2017;59(1):43–56.

[11] Gomez-Pastor R, et al. Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington's disease. Nat Commun 2017;8:14405.

[12] Qu Z, et al. Neuroprotection by heat shock factor-1 (HSF1) and trimerization-deficient mutant identifies novel alterations in gene expression. Sci Rep 2018;8(1):17255.

[13] Liangliang X, et al. Dominant-positive HSF1 decreases alpha-synuclein level and alpha-synuclein-induced toxicity. Mol Biol Rep 2010;37(4):1875–81.

[14] Kim E, et al. NEDD4-mediated HSF1 degradation underlies α-synucleinopathy. Hum Mol Genet 2016;25(2):211–22.

[15] Almeida MF, et al. Effects of mild running on substantia nigra during early neurodegeneration. J Sports Sci 2018;36(12):1363–70.

[16] Jang YC, et al. Association of exercise-induced autophagy upregulation and apoptosis suppression with neuroprotection against pharmacologically induced Parkinson's disease. J. Exerc. Nutr. Biochem. 2018;22(1):1–8.

[17] Daniele S, et al. α-Synuclein aggregates with β-amyloid or tau in human red blood cells: correlation with antioxidant capability and physical exercise in human healthy subjects. Mol Neurobiol 2018;55(3):2653–75.

[18] Zhou W, Barkow JC, Freed CR. Running wheel exercise reduces α-synuclein aggregation and improves motor and cognitive function in a transgenic mouse model of Parkinson's disease. PLoS One 2017;12(12):e0190160.

[19] Locke M, et al. Activation of heat-shock transcription factor in rat heart after heat shock and exercise. Am J Physiol 1995;268(6 Pt 1):C1387–94.

[20] Cuthbert RL, Shute RJ, Slivka DR. Skeletal muscle cold shock and heat shock protein mRNA response to aerobic exercise in different environmental temperatures. Temperature (Austin) 2019;6(1):77–84.

[21] Demirel HA, et al. Age and attenuation of exercise-induced myocardial HSP72 accumulation. Am J Physiol Heart Circ Physiol 2003;285(4):H1609–15.

[22] Javed H, et al. Plant extracts and phytochemicals targeting α-synuclein aggregation in Parkinson's disease models. Front Pharmacol 2018;9:1555.

[23] Penke B, et al. Heat shock proteins and autophagy pathways in neuroprotection: from molecular bases to pharmacological interventions. Int J Mol Sci 2018;19(1).

[24] Lee H, et al. Potentials of truffles in nutritional and medicinal applications: a review. Fungal. Biol. Biotechnol. 2020;7:9.

[25] Kurd M, et al. Effects of endurance training on hippocampus DJ-1, cannabinoid receptor type 2 and blood glucose concentration in diabetic rats. J Diabetes Invest. 2019;10(1):43–50.

[26] Zhang T, et al. Black truffle aqueous extract attenuates oxidative stress and inflammation in STZ-induced hyperglycemic rats via Nrf2 and NF-κB pathways. Front Pharmacol 2018;9:1257.

[27] Reddy VS, et al. Impact of chronic hyperglycemia on Small Heat Shock Proteins in diabetic rat brain. Arch Biochem Biophys 2021;701:108816.

[28] Giampà M, et al. Implementing complementary approaches to shape the mechanism of α-synuclein oligomerization as a model of amyloid aggregation. Molecules 2021;27(1).

[29] Alam P, et al. α-synuclein oligomers and fibrils: a spectrum of species, a spectrum of toxicities. J Neurochem 2019;150(5):522–34.

[30] Rodriguez-Araujo G, et al. Low alpha-synuclein levels in the blood are associated with insulin resistance. Sci Rep 2015;5(1):12081.

[31] Dabelea D, et al. Etiological approach to characterization of diabetes type: the SEARCH for Diabetes in Youth Study. Diabetes Care 2011;34(7):1628–33.

[32] Van Dyken P, Lacoste B. Impact of metabolic syndrome on neuroinflammation and the blood-brain barrier. Front Neurosci 2018;12:930.

[33] Matsumoto J, et al. Transmission of α-synuclein-containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease? Acta Neuropathol. Commun. 2017;5(1):71.

[34] Tian C, et al. Erythrocytic α-Synuclein as a potential biomarker for Parkinson's disease. Transl Neurodegener 2019;8:15.

[35] Martinez-Valbuena I, et al. Mixed pathologies in pancreatic β cells from subjects with neurodegenerative diseases and their interaction with prion protein. Acta Neuropathol. Commun. 2021;9(1):64.

[36] Powers SK, et al. Exercise-induced oxidative stress: friend or foe? J Sport Health Sci. 2020;9(5):415–25.

[37] Jia L, et al. Dihydromyricetin inhibits α-synuclein aggregation, disrupts preformed fibrils, and protects neuronal cells in culture against amyloid-induced cytotoxicity. J Agric Food Chem 2019;67(14):3946–55.

[38] Kuang S, et al. Effects of ginkgo biloba extract on A53T α-synuclein transgenic mouse models of Parkinson's disease. Can J Neurol Sci 2018;45(2):182–7.

[39] Abdel Mageed A, et al. Evaluation of antidiabetic potentiality of truffles and balanites aegyptiaca among streptozotocin induced diabetic rats. 2019.

[40] Atalay M, et al. Exercise training modulates heat shock protein response in diabetic rats. J Appl Physiol 1985;97(2):605–11. 2004.

[41] Purwana I, et al. HSF1 acetylation decreases its transcriptional activity and enhances glucolipotoxicity-induced apoptosis in rat and human beta cells. Diabetologia 2017;60(8):1432–41.

[42] Hemmati M, et al. Protective effects of quercetin on hyperglycemia and stress proteins expression in rats with streptozocin-induced diabetes. 2018, e64964. 15 (2.

[43] Najemnikova E, Rodgers CD, Locke M. Altered heat stress response following streptozotocin-induced diabetes. Cell Stress Chaperones 2007;12(4):342–52.

[44] Tian X, et al. Heat shock transcription factor 1 regulates exercise-induced myocardial angiogenesis after pressure overload via HIF-1α/VEGF pathway. J Cell Mol Med 2020;24(3):2178–88.

[45] Vasilaki A, et al. Adaptive responses of mouse skeletal muscle to contractile activity: the effect of age. Mech Ageing Dev 2006;127(11):830–9.

[46] Díaz-Jara E, et al. Exercise training reduces brainstem oxidative stress and restores normal breathing function in heart failure. Free Radic Biol Med 2021;172:470–81.

[47] Ye Y, et al. The effects of aerobic exercise on oxidative stress in older adults: a systematic review and meta-analysis. Front Physiol 2021;12:701151.

[48] Lin L, et al. Aerobic exercise improves type 2 diabetes mellitus-related cognitive impairment by inhibiting JAK2/STAT3 and enhancing AMPK/SIRT1 pathways in mice. Dis Markers 2022;2022:6010504.

[49] Jiang X, et al. The antidiabetic and antinephritic activities of tuber melanosporum via modulation of nrf2-mediated oxidative stress in the db/db mouse, 2018. Oxid Med Cell Longev; 2018, 7453865.

[50] Pan R, Lou J, Wei L. Significant effects of Ganoderma lucidum polysaccharide on lipid metabolism in diabetes may be associated with the activation of the FAM3C-HSF1-CAM signaling pathway. Exp Ther Med 2021;22(2):820.