



ORIGINAL ARTICLE

# Effects of Fenugreek Seed Extract and Swimming Endurance Training on Plasma Glucose and Cardiac Antioxidant Enzymes Activity in Streptozotocin-induced Diabetic Rats

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## Abstract

**Objective:** Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia condition resulting from defective insulin secretion or resistance insulin action, or both. The purpose of this study was to evaluate the effect of 6 weeks swimming training and *Trigonella foenum-graecum* seed (fenugreek) extract, alone and in combination, on plasma glucose and cardiac antioxidant enzyme activity of streptozotocin-induced diabetic rats.

**Methods:** Fifty male Wistar rats were divided into five groups: diabetic control (DC,  $n = 8$ ); healthy control (HC,  $n = 11$ ); swimming training (S,  $n = 11$ ); swimming training + fenugreek seed extract (1.74 g/kg body weight; SF1,  $n = 11$ ); and swimming training + fenugreek seed extract (0.87 g/kg body weight; SF2,  $n = 9$ ). Streptozotocin was used for the induction of diabetes. Results were analyzed using one-way analysis of variance followed by Tukey test.

**Results:** In comparison with the DC group, all groups exhibited a significant decrease in body weight ( $p < 0.05$ ), except for the HC group. SF1 and HC groups showed significant decreases in plasma glucose levels compared with the DC group ( $p < 0.05$ ). S, SF1, SF2, and HC groups showed significant elevations in cardiac antioxidant enzymes activity in comparison with the DC group.

**Conclusion:** The results indicated that the combination of endurance swimming training and fenugreek seed extract can significantly reduce the plasma glucose levels and increase cardiac antioxidant enzymes activity in diabetic rats. Our findings suggest that this combination could be useful for the treatment of hyperglycemia and cardiac oxidative stress induced by diabetes mellitus.

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## 1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or insulin action, or both [1]. Hyperglycemia may perturb cellular antioxidant defense systems and damage cells. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Oxidative stress plays an important role in the etiology of diabetes and diabetic complications [2]. Oxidative stress may constitute a focal point for multiple therapeutic interventions, and for therapeutic synergy. There is considerable evidence that oxidative stress from superoxide and other reactive oxygen species (ROS) contributes to the development of cardiovascular diseases, diabetes, and renal insufficiency [3]. Ihara et al [4] examined oxidative stress marked in diabetic rats and found increased ROS in pancreatic islets. Cells continuously produce free radicals and ROS as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase [4].

Diabetes is associated with significant oxidative stress, and oxidative damage to tissues may be a contributory factor in several diabetic complications [5]. Diabetic patients have an increased incidence of vascular disease and it has been shown that oxidative stress elevated during diabetes [6]. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance [7].

Much controversy exists concerning the effects of endurance training on the oxidative status and antioxidant defense systems of the myocardium, which may decrease, increase, or even remain unchanged [8,9]. Some controversy might arise from the different methodologies used for determinations, and differences in the models employed (running vs. swimming, rats vs. mice, male vs. female). Among various forms of treatments for diabetes mellitus, exercise and diet are of vital importance. The hypoglycemic effects of fenugreek seed have been studied in many animal model systems [10,11], as well as in humans in diabetic patients [12], but the results were controversial. In addition, fenugreek seeds have been shown to possess an encouraging antioxidant property [13] and can be a valuable candidate in the treatment and prevention of diabetes complications.

In several previous studies, the effects of training and fenugreek seed extract on the metabolic traits of diabetes were examined alone, and the results were controversial. To our knowledge, this is the first study to evaluate the effect of physical exercise and fenugreek

seed extract in combination on the cardiac antioxidants in diabetic rats.

## 2. Materials and methods

### 2.1. Animals

Fifty male Wistar albino rats, weighing 200–250 g, and averaging 12 weeks old were used in this study. They were housed in metal cages under standard laboratory condition (12:12 hours light–dark cycle and were fed regular pellets and distilled water *ad libitum*. The room had a temperature of 20–25°C, humidity of 50–60%, and average luminance of 150–200 lux in the daytime. The rats were randomly divided into five groups: (1) swimming training-fenugreek extract [1.74 g/kg body weight (BW); SF1,  $n = 11$ ]; (2) swimming training (S,  $n = 11$ ); (3) swimming training-fenugreek extract (0.87 g/kg BW; SF2,  $n = 9$ ); (4) healthy control (HC,  $n = 11$ ); and (5) diabetic control (DC,  $n = 8$ ) this group received normal saline (5 mL/kg BW). Fenugreek and saline were treated orally by gastric gavage separately. The procedures used were in accordance with the guiding principle of the responsible committee for the care and use of animals.

### 2.2. Induction of diabetes

After fasting for 12 hours, the animals received an intraperitoneal injection (60 mg/kg BW) of streptozotocin (STZ; Sigma–Aldrich, St Louis, MO, USA), diluted in 1.0 mL of sodium citrate buffer (0.1M, pH 4.5). Seven days after application of STZ and fasting for 12 hours, blood glucose was measured. Blood samples were collected by tail nipping and assessed for glucose by an electronic glucometer. Animals with levels of fasting blood glucose above 300 mg/dL were considered diabetic. Fasting blood glucose and BW were monitored at the beginning and end of the experimental period.

### 2.3. Plant material

Fenugreek seeds were purchased from the local herbal market, cleaned, dried, and finely powdered in a grinding machine. The powdered fenugreek (1.5 kg) was boiled in 15 L distilled water for 30 minutes. The decoction was cooled for 30 minutes at room temperature, then filtered twice through a coarse sieve. Finally, the filtrate was concentrated by flash evaporation at 358°C to a thick paste (total 300 g).

### 2.4. Endurance training program

The swimming training protocol was conducted in two phases, adaptation and training. The adaptation phase consisted of the first 7 days of training. On the 1<sup>st</sup> day, the animals exercised in a round plastic tank (140 cm × 60 cm × 45 cm, water temperature 34–36°C) for 10 minutes. The exercise period was

extended by 10 minutes every day until the rats could swim for 60 minutes. The training phase consisted of swimming 60 min/d, 5 d/wk for a total of 6 weeks. Swimming exercise was selected because it does not cause foot injuries, and is physically less traumatic for the animal.

### 2.5. Heart tissue and plasma preparation

At the end of the training programs, 24 hours after the last exercise-training session and 12 hours fasting, the rats were weighed, sacrificed by decapitation and then blood samples were obtained from the heart and collected into EDTA tubes. The blood was centrifuged for 10 minutes at 1500 g at 4°C. Plasma was separated carefully in tubes and then stored at -80°C until analysis.

The heart was quickly removed, washed with ice-cold saline, and blotted. After removing atria and great blood vessels, the ventricles were weighed, and the apex was cut and quickly frozen in liquid nitrogen. The duration of this process was < 2 minutes. Cardiac homogenates were prepared at 4°C. Ventricle muscle (50 mg) was homogenized on ice in 1 mL of ice-cold lysis buffer (10mM NaCl, 1.5mM MgCl<sub>2</sub>, 20mM HEPES, 20% glycerol, 0.1% Triton X-100, and 1mM dithiothreitol, pH 7.4). Tissue homogenates were centrifuged at 1500 g for 10 minutes at 4°C. The supernatants containing the cytoplasmic protein fraction were collected and protease inhibitor cocktail (104mM AEBSF, 0.08mM aprotinin, 2mM leupeptin, 4mM bestatin, 1.5mM pepstatin A, and 1.4mM E-64; P8340; Sigma—Aldrich) was added to them and stored at -80°C until use.

### 2.6. Plasma glucose measurement

Plasma glucose was measured by biochemical auto-analyzer system (BT 3000; Biotecnica, Rome, Italy).

### 2.7. Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined using a RANSOD kit (Randox Labs, Crumlin, Antrim, UK) according to the method previously described [14]. SOD activity was measured in the supernatant using a spectrophotometer at 505 nm. In this method, xanthin (0.05M) and xanthin oxidase were used to generate superoxide radicals that react with 2-(4 iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (0.025mM) to form a red formazan dye. SOD was measured by the degree of inhibition of this reaction.

### 2.8. Catalase activity

Catalase activity (CAT) was measured as previously described by Aebi [15]. The decomposition of H<sub>2</sub>O<sub>2</sub> was followed directly by the decrease in absorbance at 240 nm and 20°C. An adequate amount of supernatant (60 µL for 1.5 mg tissue wet weight) was added to a reaction mixture that contained 0.002% Triton X-100,

0.1mM EDTA, 0.5M potassium phosphate buffer, pH 7.0, and 15mM H<sub>2</sub>O<sub>2</sub> in 1 mL final volume. Activity was calculated with the initial 30 seconds decomposition rate.

### 2.9. Glutathione peroxidase activity

Glutathione peroxidase (GPX) activity was determined using a RANSEL kit (Randox Labs), according to the method previously described [16]. GPX catalyzes the oxidation of glutathione (at a concentration of 4mM) by cumene hydroperoxide. In the presence of glutathione reductase (at a concentration ≥ 0.5 units/L) and 0.28mM of NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NAD<sup>+</sup>. The decrease in absorbance at 340 nm was measured using a spectrophotometer.

### 2.10. Statistical analysis

All data are presented as means ± standard deviation and analyzed using one-way analysis of variance (ANOVA). A probability value of  $p < 0.05$  was considered statistically significant.

## 3. Results

In the beginning of the study, there was no significant difference between groups. At the end of treatment, BW was reduced in all groups, except for the HC group. BW reduction was greater in the DC and S groups than other groups ( $p < 0.05$ ; Table 1).

ANOVA results showed that there was a significant difference in plasma glucose, SOD, CAT, and GPX in between groups ( $p < 0.05$ ; Table 2).

The results showed significant differences in plasma glucose concentration between the groups: SF1 versus DC ( $p = 0.029$ ); HC versus DC ( $p < 0.001$ ); SF1 versus HC ( $p = 0.002$ ); S versus HC ( $p = 0.001$ ); and SF2 versus HC ( $p = 0.001$ ). The greatest reduction was observed in SF1 and HC groups, as compared with the DC group. In comparison with the HC group, the plasma glucose levels of all other groups were found to be significantly increased ( $p < 0.05$ ; Figure 1).

As shown in Figure 2, we found significant differences between groups for cardiac SOD activity: SF1 versus DC ( $p < 0.001$ ); S versus DC ( $p < 0.001$ ); HC versus DC ( $p < 0.001$ ); SF2 versus DC ( $p = 0.000$ ), and S versus HC ( $p = 0.002$ ; Figure 2). The maximum SOD activity was observed in the SF1 group, and it was approximately equal to the HC group.

The results indicated that there were significant differences between the groups with respect to the cardiac CAT activity; SF1 versus DC ( $p = 0.001$ ); S versus DC ( $p = 0.004$ ); HC versus DC ( $p < 0.001$ ); SF2 versus DC ( $p = 0.002$ ); S versus HC ( $p = 0.001$ ); and SF2 versus HC ( $p = 0.001$ ; Figure 3).

**Table 1.** The effect of treatment by swimming training and fenugreek seed extract on body weight (BW) after 6 weeks.

Weeks groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	<i>p</i>
SF1	205.9 ± 7.35	201.72 ± 7.45	195 ± 7.19	186.81 ± 8.91	181.72 ± 9.8	177.72 ± 11.55	0.009*
S	196.11 ± 14.95	193.33 ± 13.23	182.66 ± 10.95	171.33 ± 9.83	164.33 ± 9.38	152.22 ± 11.07	0.002*
SF2	204.12 ± 7.39	200.87 ± 7.37	195.87 ± 7.6	189.25 ± 8.76	184 ± 9.68	178.25 ± 11.94	0.007*
HC	214.4 ± 11.3	216.8 ± 10.7	219.2 ± 10.8	220.3 ± 9.9	223.4 ± 10.3	225.2 ± 11.1	0.51
DC	204.75 ± 19.94	196 ± 19.24	184.87 ± 20.43	173.12 ± 19.94	158 ± 17.89	143.75 ± 16.17	< 0.001*
<i>p</i>	0.61	0.59	0.13	0.04*	0.007*	0.002*	

\*, Statistically significant ( $p < 0.05$ ). DC = diabetic control; HC = healthy control; S = swimming training; SF1 = swimming training + fenugreek seed extract (1.74 g/kg BW); SF2 = swimming training + fenugreek seed extract (0.87 g/kg BW).

**Table 2.** The effect of treatment by swimming training and fenugreek seed extract on plasma glucose, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX).

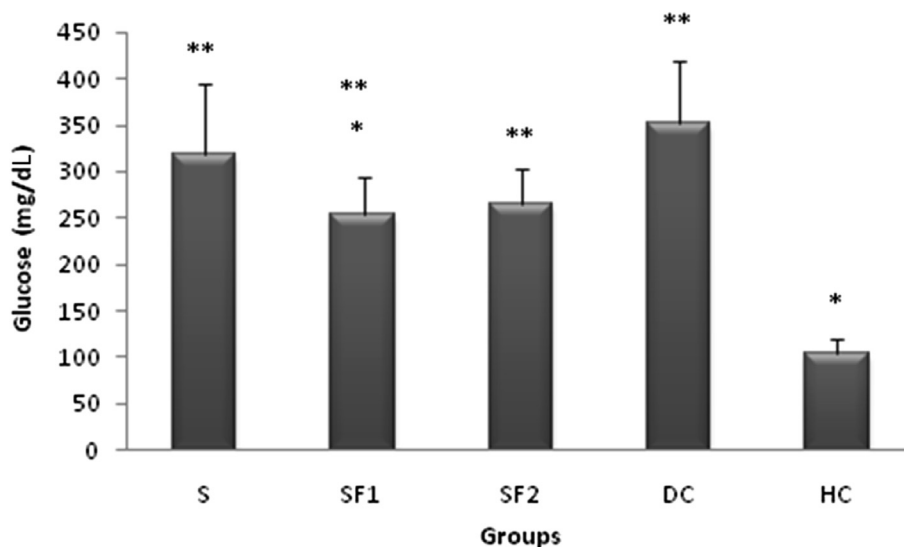
	df	F	<i>p</i>
Glucose (mg/dL)	4	23.016	< 0.001
SOD (U/mg protein)	4	23.787	0.001
CAT (U/mg protein)	4	21.379	0.005
GPX(U/mg protein)	4	6.429	0.01

There were significant differences in cardiac GPX activity between groups; SF1 versus DC ( $p = 0.008$ ); S versus DC ( $p = 0.006$ ); HC versus DC ( $p < 0.001$ ); and SF2 versus DC ( $p = 0.04$ ), but no significant difference between other groups was observed ( $p > 0.05$ ). Thus, significant increase observed at all of the groups other than the DC group ( $p < 0.05$ ; Figure 4).

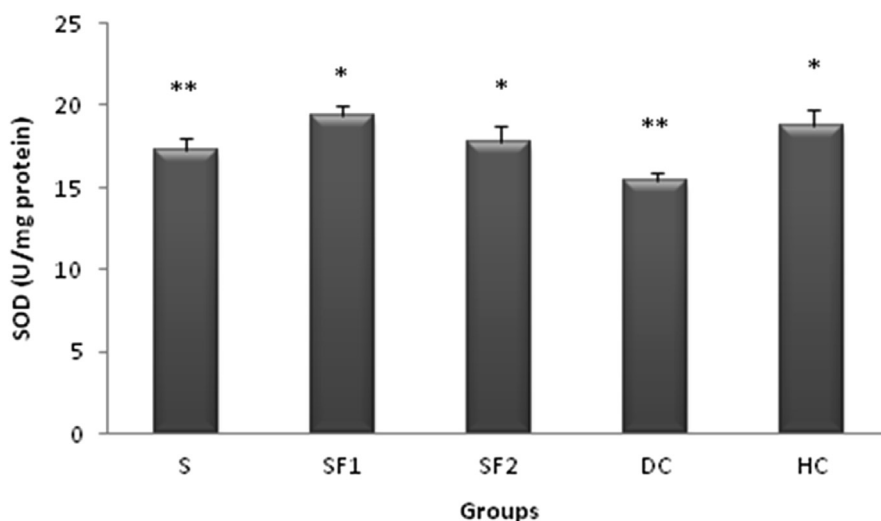
## 4. Discussion

The current study determined the effect of swimming training and fenugreek seed extract combination on plasma glucose and heart tissue antioxidants in diabetic rats. We showed that swimming training and fenugreek seeds extract combination leads to a significant reduction in plasma glucose, as compared with the diabetic control rats ( $p < 0.05$ ). Treatment with this combination also leads to a significant increase in SOD, CAT, and GPX activities in the heart tissue of diabetic rats, as compared with the diabetic control rats ( $p < 0.05$ ).

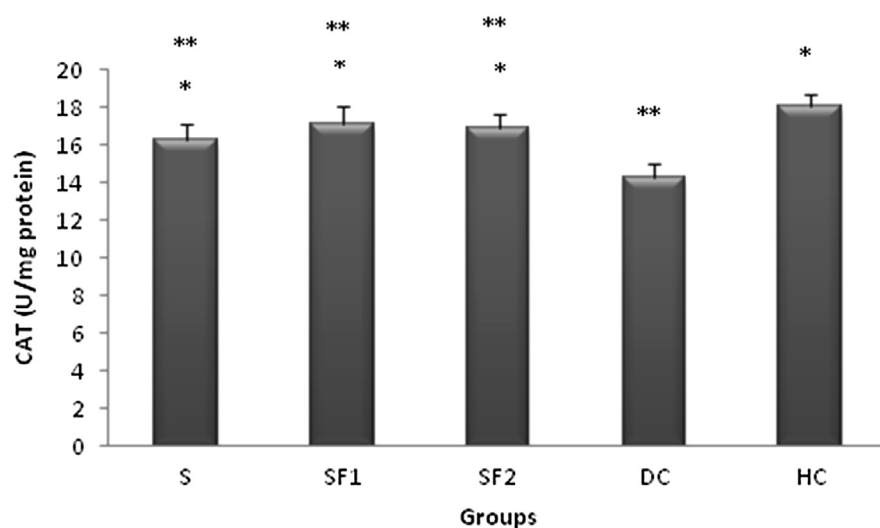
Diabetic animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby depleting the activity of the antioxidative defense system and thus promoting free radical generation. It has been recently shown that oxidative stress is responsible, at least in part, for the  $\beta$ -cell dysfunction caused by glucose toxicity. Swimming was chosen as a suitable model since it is a natural behavior of animals. The method causes less



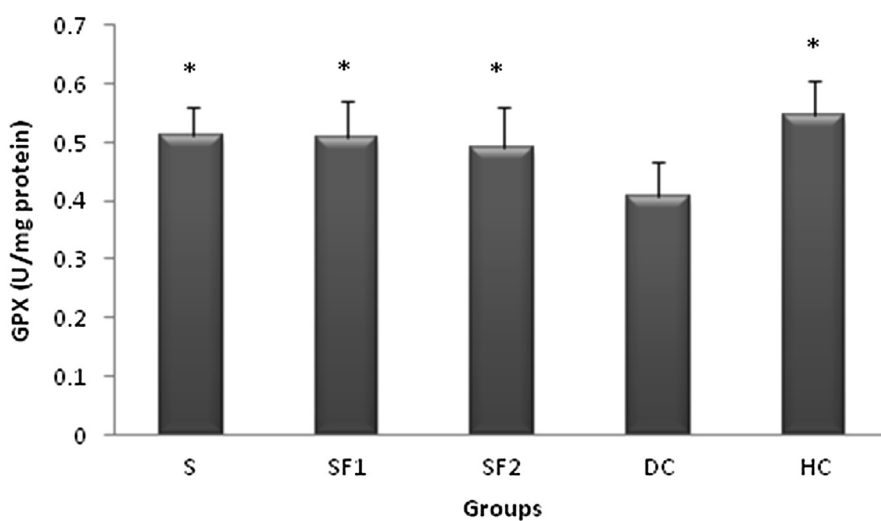
**Figure 1.** The effect of treatment by swimming training and fenugreek seed extract combination on plasma glucose. DC = diabetic control; HC = healthy control; S = swimming training; SF1 = swimming training + fenugreek seed extract (1.74 g/kg body weight); SF2 = swimming training + fenugreek seed extract (0.87 g/kg body weight). \*Statistically significant versus DC group. \*\*Statistically significant versus HC group.



**Figure 2.** The effect of treatment by swimming training and fenugreek seed extract on heart superoxide dismutase (SOD) activity. Abbreviations are as Figure 1. \*Statistically significant versus DC group. \*\*Statistically significant versus HC group.



**Figure 3.** The effect of treatment by swimming training and fenugreek seed extract on heart catalase (CAT) activity. Abbreviations are as Figure 1. \*Statistically significant versus DC group. \*\*Statistically significant versus HC group.



**Figure 4.** The effect of treatment by swimming training and fenugreek seed extract on heart glutathione peroxidase (GPX) activity. Abbreviations are as Figure 1. \*Statistically significant versus DC group.



mechanical stress and injury, and leads to a better redistribution of blood flow among tissues without significant variations in cardiac output and heart rate, which in turn may minimize the magnitude of injury caused due to the generation of ROS [17]. In agreement with our results, previous study has shown that endurance exercise decrease serum glucose in diabetic animals and humans [18]. Since skeletal muscle responds to endurance exercise via a series of structural and functional adaptations, the increased responsiveness to insulin induced by swimming exercise in rat skeletal muscle may result partly from modulation of the insulin signaling pathway at different molecular levels. In particular, the insulin receptor substrate/phosphatidylinositol-3-kinase pathway may be involved in the activation of glucose transport and glycogen synthesis in muscle, and an increase in this association in the muscle of trained animals and humans may have an important role in the responsiveness to insulin. Hyperglycemia increases the production of ROS through protein glycation and patients with type 1 diabetes mellitus have higher plasma concentrations of free radicals than healthy individuals [19]. In this way, increased glucose level is an important factor implicated in the development of the diabetes-associated complications. Previous researchers analyzed the effects of exercise training on myocardial antioxidant systems, but the overall results are controversial [9,20,21]. To our knowledge, no study has used the combination of endurance swimming training and fenugreek seed extract in combination on plasma glucose and antioxidants in heart tissue of diabetic rats.

Several studies have shown changes in antioxidant activity in various tissues follow different patterns, which are not yet known. Previous studies have shown increased antioxidant enzymes after endurance training [22–25]. These findings are consistent with our results, but we used combinations of endurance swimming training and fenugreek seed extract, and observed the greater elevation in antioxidant enzymes levels, as compared with those treated with swimming training or extract alone. In contrast to our results, some studies have reported decreased cardiac antioxidant enzymes after endurance training [26,27]. Overall, inconsistent results could be due to some factors such as age, sex and animal breed, different techniques used in the assessment, type of exercise, duration and intensity of exercise, and the tissue used.

Several mechanisms have been suggested to explain the hypoglycemic action of fenugreek. These include modulation of insulin secretion, insulin-mimetic effect, inhibition of intestinal glucosidase activity, and the presence of coumarins and the alkaloid trigonelline [28]. Therefore, fenugreek may be a secondary mechanism for hypoglycemic and antioxidative stress effect. Overall, the present study provides evidence that the combination of endurance training and fenugreek are capable of influencing plasma glucose and antioxidant

enzymes in cardiac muscle of diabetic rats. We showed that this combination increases the extent of antioxidant enzymes in cardiac muscle such that it can lead to decreases in apoptosis in cardiac cells.

## Conflicts of interest

The authors declare that they have no competing interests.

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