emergencias 2014;2:95-101

Original Article

The effect of Swimming training and Fenugreek seed Supplementation on Plasma glucose and Heart tissue Antioxidants in Diabetic rats

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Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defective insulin secretion or resistance insulin action, or both. Research question: The purpose of this study was to evaluate the effect of 6 weeks swimming training and Trigonella foenum-graecum seed (fenugreek) extract supplementation on plasma glucose and cardiac tissue antioxidants in diabetic rats. Type of study: For this purpose, 50 male wistar rats were divided into five groups; Diabetic control (DC, n=10), Healthy control (HC, n=10), Swimming training (S, n=10)), one group of Swimming training + Fenugreek seed extract (1.74 g/kg BW) (SF1, n=10), and Swimming training + Fenugreek seed extract (0.87 g/kg BW) (SF2, n=10). Methods: In this study was used streptozotocine (STZ) for induction of diabetes. Results were analyzed using the one-way ANOVA followed by a Tukey test. Significance level was 0.05. Results: All of the groups except HC group exhibited a significant decrease in body weight ($P<0.05$). SF1 and HC groups have shown a significant decrease in plasma glucose than DC group ($P<0.05$). S, SF1, SF2 and HC group have shown significant increase in cardiac antioxidant enzymes than DC group. Conclusions: The present results indicate that the combination of endurance swimming training and fenugreek seed extract can useful for treating metabolic diseases related to obesity and diabetes.

Keywords: **Endurance swimming training, Fenugreek seeds extract Heart antioxidants**

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defective insulin secretion or resistance insulin action, or both (4). Hyperglycemia may perturb cellular antioxidant defense systems and damage cells. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Oxidative stress plays an important role in the etiology of diabetes and diabetic complication (3). 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 ROS contributes to the development of cardiovascular diseases, diabetes, and renal insufficiency (8). Ihara et al (2000) (10) examined oxidative stress marked in diabetic rats and found increased reactive oxygen species (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.

Diabetes is associated with significant oxidative stress, and oxidative damage to tissues may be a contributory factor in several diabetic complications (12). Diabetic patients have an increased incidence of vascular disease and it has been shown that oxidative stress elevated during diabetes (15).

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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. Much controversy exists concerning the effects of endurance training on the oxidative status and antioxidant defense systems of the myocardium as decrease, increase, or even remain unchanged (for a comprehensive review see Ji L.L review, 1999) (11). 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 diabetic mellitus, exercise and diet are of vital importance. The hypoglycemic effects of Fenugreek seed has been studied in many animal model systems (14), as well as in humans in diabetic patients (19, 20) but 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.

as regards previous studies concern only the effect of training or extract in metabolic disease and suggest conflict results but This study is the first study that evaluates the effect of physical exercise and fenugreek seeds extract combination on cardiac antioxidants in diabetic rats.

We indicate whether the institutional and national guide for the care and use of laboratory animals was followed

Materials and methods

Animals

Fifty male wistar albino rat, weighting 200-250 g, and averaging 12 weeks old were used in this study. They house in metal cage under standard laboratory condition (12:12-h 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 illuminance of 150–200 lux in the daytime. the rats were randomly divided into five groups: 1: swimming training-fenugreek extract (1.74 g/kg BW) (SF1) (n=11), 2: swimming training (S) (n=11), 3: swimming training-fenugreek extract (0.87 g/kg BW) (SF2) (n=9), 4: health control (HC), 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 followed were in accordance with the guiding principle of the responsible committee for the care and use of animals.

Induction of diabetes

After fasting for 12 hours, the animals received an intraperitoneal injection (60 mg/kg body weight) of streptozotocine (STZ, Sigma, St. Louis, USA), diluted in 1.0 ml of sodium citrate buffer (0.1 M, 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 body weight were monitored at first and end in the experimental period.

Plant material

Fenugreek seeds were purchased from the local herbal market, cleaned, dried and finely powdered in a grinding machine.

Extraction of aqueous plant material

1.5 kg of powdered Fenugreek seeds were boiled in 15000 ml distilled water for 30 mines. Then, the decoction was cooled for 30 mines at room temperature. Next, the cooled decoction was filtered through a coarse sieve twice. Finally, the filtrate was concentrated by flash evaporation at 358 C° to a thick paste (totally 300 g).

Endurance training program

Swimming training protocol was conducted in 2 phases, adaptation and training. The adaptation phase consisted of the first 7 days of training. On the first day, the animals exercised in a round plastic tank (140x60x45 cm and water temperature about 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/day, 5 days/week for a total of 6 weeks.. Swimming exercise was selected because it did not cause foot injuries, and is physically less traumatic for the animal.

Heart tissue and plasma preparation

At the end of the training programs, 24 h after the last exercise-training session and 12 h fasting, the rats were weighed, sacrificed by decapitation and then Blood samples were obtained from heart of rats, whole blood were collected in an EDTA tube. The blood was centrifuged for 10 minutes at 3000 rpm in 4 °C. Plasma was separated carefully in a number of ependroof tubes and then stored at-80°C until analyze time.

The heart was quickly removed, washed with ice-cold saline, and blotted. After the atria and great blood vessels were trimmed, the ventricles were weighed, and the apex was cut and quickly frozen in liquid N2. Duration of the process was less than 2 min. Cardiac homogenates were prepared at 4ºC. fifty milligrams of ventricle muscle were homogenized on ice in 1 ml of ice-cold lysis buffer (10 mM NaCl, 1.5 mM MgCl2, 20 mM HEPES, 20% glycerol, 0.1% Triton X-100, 1 mM dithiothreitol, pH 7.4). Tissue homogenates were centrifuged at 3,000 rpm for 10 min at 4°C. The supernatants contained the cytoplasmic protein

fraction were collected and protease inhibitor cocktail (104 mM AEBSF, 0.08 mM aprotinin, 2 mM leupeptin, 4 mM bestatin, 1.5 mM pepstatin A, and 1.4 mM E-64) (P8340, Sigma-Aldrich, St Louis, MO) was added to them and stored at 80 ºC until use.

Plasma glucose measurement

Plasma glucose was measured by biochemical auto analyzer system (BT 3000, USA).

SOD Activity

Superoxide dismutase (SOD) activity was determined using a RANSOD kit (Randox labs. Crumlin, UK) according to Delmas-Beauvieux, M.C., Peuchant, E., Dumon, M.F., Receuver, M.C., Le Bras, M., Clerc, M, 1995 (6). SOD activity was measured at 505 nm on a spectrophotometer on supernatant. In this method, xanthin and xanthin oxidase were used to generate superoxide radicals that react with 2-(4 iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (ITN) to form a red formazan dye. Concentrations of substrates were 0.05 mmol•L-1 for xanthin and 0.025 mmol•L-1 for INT. SOD was measured by the degree of inhibition of this reaction.

CAT Activity

Catalase activity (CAT) was measured as previously described by Aebi H, 1984 (1). The decomposition of H2O2 was followed directly by the decrease in absorbance at 240 nm and 20 ºC. Pr. The adequate amount of supernatants (60 µL equivalent to 1.5 mg tissue wet weight) was added to a reaction mixture that contained 0.002% Triton X-100, 0.1 mm EDTA, 0.5 m potassium phosphate buffer, pH 7.0, and 15 mm H2O2 in 1 mL final volume. Activity was calculated with the initial 30s decomposition rate.

GPX Activity

Glutathione peroxidase (GPX) activity was determined using a RANSEL kit (Randox labs.), according to the method of Paglia, D.E., Valentine, W.N, 1967 (16). GPX catalyses the oxidation of glutathione (at a concentration of 4 mmol•L-1) by cumene hydroperoxide. In the presence of glutathione reductase (at a concentration ≥0.5 units/L) and 0.28 mmol/L of NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NAD+. The decrease in absorbance at 340 nm was measured using a spectrophotometer.

Statistical Analysis

Results are presented as means ± standard deviation and were analyzed using One- Way ANOVA with Tukeys Post Hoc comparisons. A probability of 0.05 or less was considered as statistically significant.

Results

ANOVA Results shown that there was significant difference in plasma glucose, SOD, CAT and GPX in DC than other groups (P<0.05) (Table 1).

Plasma glucose

The results showed that there were significant differences between the groups in plasma glucose concentration; SF1 than DC (P=0.029), HC group than DC group (P=0.000), SF1 group than HC group (P=0.002), S group than HC group (P=0.001), SF2 group than HC group (P=0.001). Therefore, the more reduction was in SF1 and HC group than other groups compared to DC group, but there wasn't significant difference between other groups (P>0.05). There was also significant increase at all of the groups than HC (P<0.05) (Figure 1).

Antioxidants activity

SOD activity

The results have showed that there were significant differences between the groups in cardiac SOD; SF1 group than DC group (P=0.000), S group than DC group (P=0.000), HC group than DC group (P=0.000), SF2 group than DC group (P=0.000), S group than HC group (P=0.002). But there weren't significant difference between other groups (P>0.05). Thus, there was more increase in SF1, SF2, and HC than S group compared with DC group (Figure 2).

CAT activity

The results have showed that there were significant differences between the groups in cardiac CAT; SF1 than DC (P=0.001), S group than DC group (P=0.004), HC group than DC group (P=0.000), SF2 group than DC group (P=0.002), S group than HC group (P=0.001), SF2 group than HC group (P=0.001). Thus, significant increase observed at all of the groups' than DC group that it was more in SF1, SF2 and HC (P<0.05) and there was significant decrease at all of the groups' than HC (Figure 3).

GPX activity

The results have showed that there were significant differences between the groups in cardiac GPX; SF1 than DC (P=0.008), S group than DC group (P=0.006), HC group than DC group (P=0.000), SF2 group than DC group (P=0.04), but there wasn't significant difference between other groups (P>0.05). Thus, significant increase observed at all of the groups' than DC group (P<0.05) (Figure 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 show swimming training and fenugreek seeds extract combination lead to significant decrease in plasma glucose than diabetic control ($P<0.05$) and lead to significant increase in SOD, CAT and GPX in heart tissue of diabetic rats than diabetic control ($P<0.05$).

Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby deplete the activity of anti-oxidative defense system and thus promote free radicals generation. Oxidative stress recently been shown to be responsible, at least in part, for the β-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 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 (18). Previous studies have shown endurance exercise decrease serum glucose in diabetic animals and human (7) that is consistence with our results. Since, skeletal muscle responds to endurance exercise via a series of structure 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 IRS/IP3-kinas 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 human may have an important role in the responsiveness to insulin. Hyperglycemia increases the production of reactive oxygen species through protein glycation and patients with type 1 diabetes mellitus have higher plasma concentrations of free radicals than healthy individuals (5). In this way, increased glucose level is an important factor implicated with the development of the diabetes-associated complications. Previous researches' analyzed the effects of exercise training on myocardial antioxidant systems but the overall results are controversial (for a comprehensive review see references of Ji, L.L. 1999 and Atalay, M. and Sen, C.K, 1999) (2,11). But to our knowledge no study has used the combination of endurance swimming training and fenugreek seed extract on plasma glucose and antioxidants in diabetic rats' heart tissue.

Previous studies have shown increases (9) in antioxidant enzymes after endurance training that are consistent with our results but we used the combination of endurance swimming training and fenugreek seed extract. Differences in these factors might explain the controversial results about antioxidants activity in response to training. Results have shown that changes in antioxidant activity in various tissues follow different pattern, that the pattern of these changes is not yet known. The overall, Inconsistent results in this studies than other studies could be due to some factors such as Age, sex and animals race, different techniques in the assessment, type of exercise, duration and intensity of exercise and the tissue that is used.

Several mechanisms have been suggested to explain the hypoglycemic action of Fenugreek. These include modulation of insulin secretion, insulinomimetic effect, inhibition of intestinal glucosidase activity and the presence of coumarins and the alkaloid trigonelline (17). Therefore fenugreek may be a secondary mechanism for hypoglycemic and anti-oxidative stress effect.

Conclusion

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

Acknowledgements

All authors have made significant contributions to the work that is reported. We thank for Islamic Azad University South Tehran Branch for their great assistant and financial support in this paper.

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ISSN: 1137-6821
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Table1: the effect of treatment by swimming training and fenugreek seed extract on plasma glucose, SOD, CAT and GPX

	DF	F	Sig
Glucose (mg/dl)	4	23.016	000
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

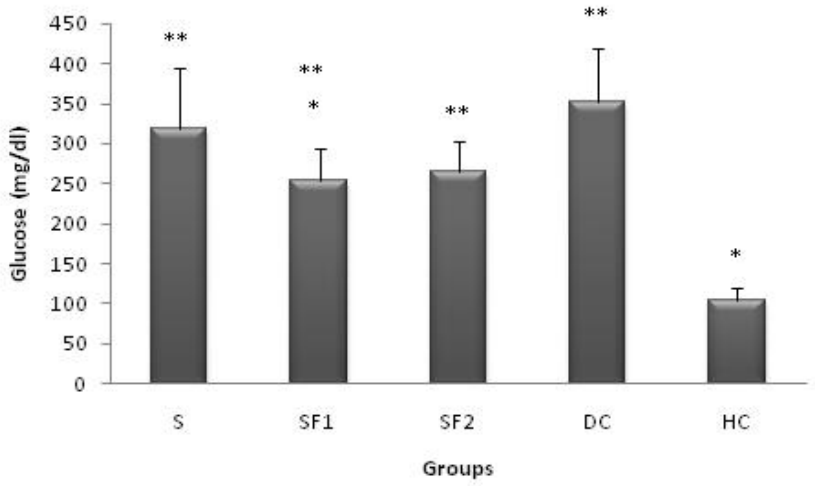

Figure1: The effect of treatment by swimming training and fenugreek seed extract combination on plasma glucose.
SF1: Swimming training + fenugreek seeds extract 1.74 g/kg body weight, S: Swimming training, SF2: Swimming training + fenugreek seeds extract 0.87 g/kg body weight, HC: Health control, DC: Diabetic control. * Significant decrease in SF1 and HC groups than DC. ** Significant increase in all of groups than HC.

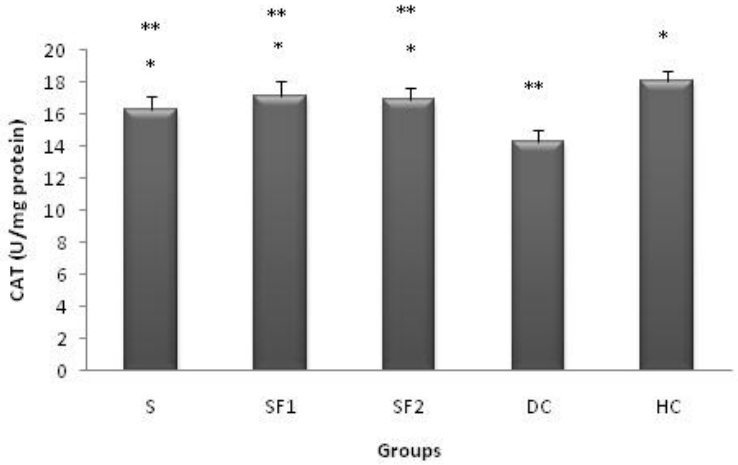

Figure 2: the effect of treatment by swimming training and fenugreek seed extract on Heart SOD
SF1: Swimming training + fenugreek seeds extract 1.74 g/kg body weight, S: Swimming training, SF2: Swimming training + fenugreek seeds extract 0.87 g/kg body weight, HC: Health control, DC: Diabetic control. * Significant increase in SF1, SF2 and HC than DC. ** Significant decrease in S and DC than HC.

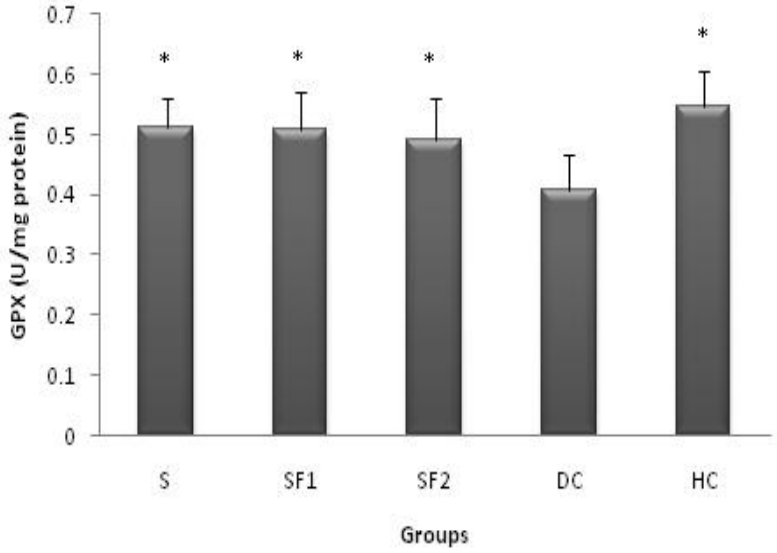

Figure 3: the effect of treatment by swimming training and fenugreek seed extract on Heart CAT
SF1: Swimming training + fenugreek seeds extract 1.74 g/kg body weight, S: Swimming training, SF2: Swimming training + fenugreek seeds extract 0.87 g/kg body weight, HC: Health control, DC: Diabetic control. * Significant increase in all of the groups than DC. ** Significant decrease in all of the groups than HC.

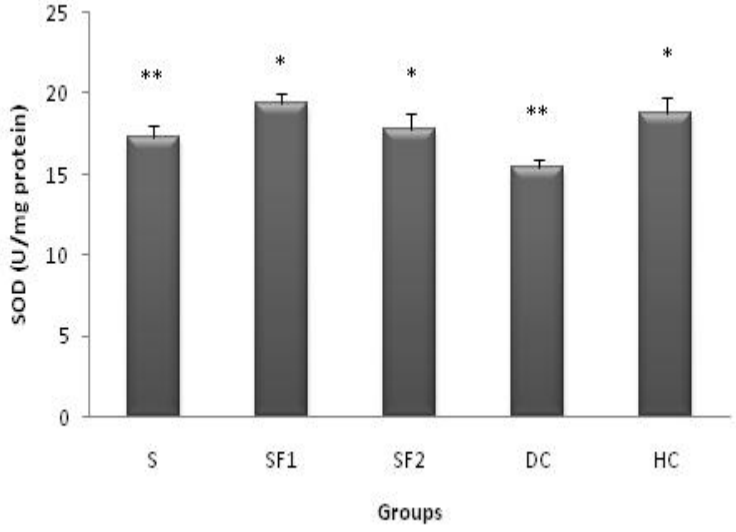

Figure 4: the effect of treatment by swimming training and fenugreek seed extract on Heart GPX
SF1: Swimming training + fenugreek seeds extract 1.74 g/kg body weight, S: Swimming training, SF2: Swimming training + fenugreek seeds extract 0.87 g/kg body weight, HC: Health control, DC: Diabetic control. * Significant increase in all of the groups than DC.