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ORIGINAL ARTICLE

The Effect of Aerobic Training program Combined with cranberry Consumption on serum IL-17 and Cortisol in Female Diabetes rats

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ABSTRACT

The present study aims at investigating the effect of aerobic exercise, together with consuming cranberry, on serum levels of interleukin (IL)-17 and cortisol in streptozotocin-induced diabetic rats. In so doing, 50 2-month-old female Wistar rats weighing between 200 and 300 g. were randomly selected from among those bred in Laboratory Animals Breeding Center, Razi Vaccine and Serum Research Institute. The rats were divided into five groups as follows: healthy control group, diabetic control group, diabetic experimental group receiving cranberry, diabetic experimental group receiving aerobic exercise, diabetic experimental group receiving both cranberry and aerobic exercise. Familiarization procedure was done prior to the training program which included four weeks of running –five days per week. In the first week, rats ran on a treadmill for 10 min (14 m per min and with the slope of zero); in the second week, rats ran on a treadmill for 20 min (14 m per min and with the slope of zero); in the third week, rats ran on a treadmill for 30 min (14 m per min and with the slope of zero); in the fourth week, rats ran on a treadmill for 40 min (14 m per min and with the slope of zero). Serum levels of interleukin (IL)-17 and cortisol were measured and analyzed using commercial ELISA kit. Kolmogorov-Smirnov test was used to examine the normal distribution of data. As for data description, descriptive statistics, central tendency and distribution indices were applied one -way ANOVA and gyms howel Test were also used through SPSS 18 at the level of significance 0.05. the study yielded the result that aerobic exercise, together with consuming cranberry has a significant effect on serum levels of interleukin (IL)-17 and cortisol in streptozotocin-induced diabetic rats.

Key words: *cranberry, IL 17, cortisol, and streptozotocin-induced diabetic rats.*

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INTRODUCTION

Diabetes is a multifactorial disease impairing glucose homeostasis in the body. Given the critical, constant need for glucose in tissues, e.g. those in the brain, human body always maintains the glucose level within a fixed range. In patients with diabetes, as sensitivity of cells to insulin varies, glucose homeostasis is impaired leading to chronic and acute hyperglycemia which ultimately gives rise to atherosclerotic alterations, micro and macro vascular complications in patients. In resting position, glucose uptake in the skeletal muscle takes place mainly by insulin stimulation. In fact, it is the glucose transporter to skeletal muscle by Glut4 transported by insulin. This phenomenon is impaired in people with diabetes, who perform aerobic exercise leading to muscle contraction through activation of protein kinase connected to Amp causing the Glut4 displacement. Therefore, aerobic exercise can provide Glut4 displacement and increase glucose uptake in such patients [1]. Treatment of acute and chronic complications of the disease imposes enormous expenses annually to the developed and developing countries. Accordingly, this study intended to seek a solution through which diabetes can be controlled, minimizing the costs of treatment. Diabetes is caused by the inability of muscle cells to respond to insulin, where hereditary and environmental factors are involved. Diabetes can be aggravated by obesity and inactivity with increasing

age. Hence, one of the useful ways to improve the patients' health status is physical activity [2]. The previous studies found that exercise is effective in improving diabetes. Aerobic exercise is accompanied by reducing fat oxidation and transfer toward higher oxidation of carbohydrates at any exercise intensity among people with diabetes. Even aerobic exercise can immediately improve insulin performance and remain active from 2 to 72 hours [3]. Although considerable attention has been paid to metabolic effects of exercise on obesity, diabetes and insulin resistance, there is little information about the effect of physical exercise on insulin resistance-causing mechanisms such as cytokines and their interrelationship [4].

However, the immune system is closely related to a variety of diseases, involving many cells with cytokine receptors. Cytokines are secreted from the innate and adaptive immunity cells, mediating many of the actions of these cells. In recent years, several cytokines have been identified with certain characteristic such as IL-17 and IL -18. IL-17 is a proinflammatory cytokine secreting other cytokines including IL18, IL6, IL2, TNF-α [5]. This cytokine is so crucial that researchers have shown that an increased level of IL-17 is associated with many diseases [6]. By increasing the buildup of neutrophils in the peripheral tissues, IL-17 escalates inflammation and strengthens the defense against extracellular bacteria [7].

Cortisol is a key steroid hormone regulating the cardiovascular functions, immunological and metabolic homeostasis. Moreover, it can accelerate the gluconeogenesis of proteolysis and lipogenesis [8]. Researchers have reported that factors such as rigorous exercise, changes in plasma and blood volume, and psychological pressures caused by exercise [9] can affect serum cortisol concentration in the athletes. In addition, it has been found that such hormones increase in athletes, thus impairing immunity [10].

Another method of treatment involves herbal medicines considered an important natural resource in the world applied by humans since a very long time ago [11]. One of the important medicinal plants is bilberry from Ericaceae family. Bilberry is highly adopted in the Iranian medicine, which has been traditionally popular as an anti-diabetes drug. According to precious studies, the leaves and fruits of the plant can be applied for treating diabetes. Moreover, the extract or powder prepared from aerial parts (especially leaves) can reduce blood glucose and triglycerides in diabetic animals. Research also shows the effect of bilberry in reducing blood glucose and lipids in patients with type 2 diabetes [13].

Given the above fact, it appears that the hypoglycemic effect by physical activity and bilberry alone has been revealed. However, there is little information on the simultaneous effects of aerobic exercise over a short period and bilberry on diabetes treatment. Hence, it is essential to examine the simultaneous effect of the two factors. Given that the results of this study could provide the diabetic patients with an in-depth insight concerning the effect of bilberry and aerobic training on oxidative stress, it was critical to further investigate the issue and employ bilberry as a natural supplement to improve diabetes.

The researcher hopes to address this issue so as to fulfill the needs of those interested in sports science and physical education still appealing to any scholar the boundaries to break through unknown boundaries. The main objective was to expand the knowledge of the sports community.

MATERIAL AND METHODS

The population of this experimental study comprised the entire female Wistar rats aged 2 months in the weight range of 200 to 300 g at Razi Vaccine and Serum Research Institute (RVSRI) for Proliferation of Laboratory Animals. A total of 50 rats were randomly selected as sample. At first, 4 to 6 sample rats were kept freely with food and water in laboratory experiments at Rojan Industrial Park at temperature of 20-23 degrees Celsiu, 12-hour light-dark cycle (starting at 9 am and ending at 9 pm) in each breeding cage. All the behavioral tests were performed from 10 am to 4 pm. One week after adaptation to the laboratory environment, the diabetes process started followed by trials and consumption of bilberry a week after induction of diabetes. The rats were randomly divided into five groups: normal control and diabetic healthy, which did not take bilberry nor exercise. The diabetic group performed aerobic exercise 5 days a week at 6 pm.

The group freely receiving bilberry mixed with rat chow during the day did not perform any exercise. The third group freely consumed bilberry mixed with rat chow during the day and performed aerobic exercise 5 days a week at 6 pm. It should be noted that for preparation of the food containing bilberry upon scientific confirmation, the powder was first obtained and then the shoot was grounded at a relative weight ratio of 6.25% with standard rat diet and powder (14). According to current protocols, the appropriate amount of streptozotocin in diabetic induction (destruction of pancreatic islet cells) is equivalent to 25 to 60 micrograms per kilogram of body weight. To build a proper solution, 1 ml of citrate buffer was solved in 10 mM of regular stock solution of 1 g per 50 ml buffer solution, which was prepared freshly and is kept at 20°C. Based on the murine weights, the suitable range was between 25 and 60 micrograms of toxin injection for each rat.

Familiarizing the rats with treadmill

During the 4-day familiarization, frequent and short training courses were adopted. Each stage involved 5 minutes of jogging at a speed of 14 meters per minute once a day. After the familiarization period, 1 day of rest was given to the animals followed by the training period for included samples. Stimuli such as sound, hitting the rear of the animal, and if necessary, electric shock were adopted to force the animals to keep jogging. The main training program for the animals involved 10 minutes in the first week, 20 minutes i the second week, 30 minutes in the third week and 40 minutes in the fourth week at a speed of 14 meters per minute with zero slopes on the treadmill.

At the end of the study, the animals were sacrificed and their livers were removed. The night before taking blood samples the animals were deprived of food for 12 hours just to drink water. For sampling, the animals were anesthetized in the chloroform chamber. The blood samples transported immediately to the laboratory. For isolation of serum, the samples were centrifuged for 5 minutes at 3000 RPM. The serum obtained from each sample was poured two distinct micro-tubes at the required amount. A series of micro-tubes containing serum samples were immediately kept in the laboratory at a temperature of -80 C until the ELISA kits for IL-17 arrived.

Finally, descriptive statistics (central tendency and dispersion) were employed to describe the data. Moreover, the ANOVA and Haul's post hoc test were adopted at significant level ($\alpha<0.05$) using SPSS (version 18) and MS Excel.

RESULTS

The results showed that the data distribution was normal. Furthermore, an aerobic exercise course with bilberry consumption together with serum levels of IL-17 F = 4,35 = 0.000 and cortisol F = 4,35 = 0.000 in streptozotocin-induced diabetic rats had a significant impact.

Table 1: Data of Gyms Hole test of IL-17

Comparison between groups	Delta of mean	Sig
Normal - Diabetic	49.89	*p = 0.006 **
Normal – Diabetic - cranberry	71.78	*p = 0.001 **
Normal – Diabetic - Exercise	70.70	*p = 0.001 **
Normal – Diabetic- cranberry - Exercise	56.42	*p = 0.002 **
Diabetic – Diabetic - cranberry	21.96	*p = 0.006 **
Diabetic – Diabetic - Exercise	120.53	*p = 0.000 **
Diabetic – Diabetic - cranberry - Exercise	106.25	*p = 0.000 **
Diabetic - cranberry - Diabetic - Exercise	142.49	*p = 0.000 **
Diabetic - cranberry - Diabetic – cranberry - Exercise	128.21	*p = 0.000 **
Diabetic - Exercise - Diabetic – cranberry - Exercise	14.28	*p = 0.129*

Based on the table, there was no significant difference between the diabetic and diabetic exercise group and the diabetic and aerobic exercise taking bilberry, while there was a significant difference between the other groups. This shows that the results of Haul's test Games were lower than the inter-group difference between these groups.

Table 1: Data of Gyms Hole test of Cortisol

Comparison between groups	Delta of mean	Sig
Normal - Diabetic	2.17	*P = 0.000 **
Normal – Diabetic - cranberry	7.67	*P = 0.000 **
Normal – Diabetic - Exercise	5.20	*P = 0.000 **
Normal – Diabetic- cranberry - Exercise	4.84	*P = 0.000 **
Diabetic – Diabetic - cranberry	5.49	*P = 0.000 **
Diabetic – Diabetic - Exercise	3.03	*P = 0.000 **
Diabetic – Diabetic - cranberry - Exercise	2.67	*P = 0.000 **
Diabetic - cranberry - Diabetic - Exercise	2.64	*P = 0.000 **
Diabetic - cranberry - Diabetic – cranberry - Exercise	2.82	*P = 0.000 **
Diabetic - Exercise - Diabetic – cranberry - Exercise	0.36	*P = 0.004 **

The results of Games Haul's test showed that there were significant differences between all pairs of groups. This shows that the results of Haul's test Games were lower than the inter-group difference between these groups.

DISCUSSION

The results showed that supplements and aerobic exercise left a significant effect on serum levels of IL-17 in female rats with streptozotocin-induced diabetes. The results in this section were consistent with those obtained by was Alizadeh *et al*. [15] who examined changes in inflammatory markers and muscle damage in rats after 8 weeks of intense aerobic exercise and intake of omega-3. The results showed significant differences in serum levels of IL-17 between the control and other groups, between exercise group and supplement group, and between supplement group and supplement/exercise group. Moreover, the level of IL-17 was higher in the supplement-exercise group than in the supplement group.

Moreover, Golzari *et al.* [16] examined the effect of eight-week combined exercise training on plasma levels of IL-17. The results showed that production of plasma IL-17 and mononuclear cells significantly decreased in the exercise-group. Similarly, Anderson *et al*. (2010) studies the response of inflammatory and anti-inflammatory cytokines in two 90-minute soccer matches with 72 hours of active and passive recovery among the elite female soccer players [17]. Their findings showed that IL-17 in both groups significantly increased immediately after the first match. In fact, 21 hours after the first match, the levels of cytokine in the active recovery group slightly increased, while it decreased in the passive recovery group. Moreover, 45 hours after the first match, the amount of IL-17 reduced for 69 hours, but it did not reach the baseline.

Finally, there was a significant increase in IL-17after the second match. However, the results of the current study were inconsistent with those obtained by Kunda *et al*. [18] who showed that IL-17 levels was influenced through physical exercise. The reason could be attributed to higher level of physical fitness in subjects as compared those selected in the current study, thus leading to lower cytokine response. Furthermore, Cortisol suppresses the cytokine activity by stimulating the HPA axis through proinflammatory cytokines. As observed in the current study showed, the serum cortisol levels significantly changed after the exercise course. Based on previous studies on rats, it was concluded that bilberry extract can reduce blood glucose and triglyceride levels in diabetic animals. Clinical research also shows the effect of bilberry in reducing blood glucose and lipids in patients with type 2 diabetes.

In this experiment, the effective mechanism in diabetes involves bilberry as a factor inhibiting glucose from the intestines to the blood. Moreover, antioxidants in bilberry can curtail the blood triglyceride levels. Variations of IL-17 due to endurance exercise may be associated with further increase in the levels of glucocorticoids, especially cortisol, which is mainly because of greater production of lactate having a direct relationship with cortisol. The mechanisms of IL-17 in this regard can be the growth inhibition of fat cells and increase differentiated lipolysis of fat cells as well as regulation of inflammatory responses in adipocytes, which is an additional function of cytokines.

Another finding showed that supplements and aerobic exercise left a significant effect on serum levels of plasma cortisol in female rats with streptozotocin-induced diabetes. Cortisol is a key glucocorticoid secreted by the adrenal gland, i.e. a metabolic hormone enabling the body to counteract stress owing to higher gluconeogenesis and blood glucose levels. Cortisol release is influenced by many factors such as changes in environmental conditions and diet, physiological stress and training conditions [7]. It is expected that physical activity in the long run adjust stress and exercise pressure by reducing the sensitivity of glucocorticoids. Kramer et al. showed that participation in ultramarathon cycling (160 km) led to increased levels of cortisol [7]. More massive exercise protocols are accompanies with significant effect on catabolic hormones such as cortisol at moderate to severe intensities in larger muscle masses. Concentration of cortisol raises in response to vigorous prolonged exercise reacting to hypoglycemia, i.e. the resting levels of cortisol can reflect the amount of exercise and suitable workload pressure [7]. Hence, characteristics exercise training applied (intensity) in this study protocol was adequate to yield significant changes in plasma cortisol levels.

In another study, it was revealed that a session of aerobic exercise decreased blood cortisol concentrations immediately after workout. The average concentration of the hormone decreased at the end of practice as compared to resting position [19]. The failure of consistency between the findings and those of previous studies can be attributed to the type of and style of physical exercises. For justifying the concentration variations in resting levels of cortisol after physical activity, there are different mechanisms such as hypothalamic-pituitary stimulation of in the sympathetic nervous system and PH central temperature changes, adrenal ACTH changes, and secretion of lactate and hypoxia. It seems that increased volume of exercise can reduce endurance performance, thus curtailing the secretion of catecholamines and sympathetic activity. It has been reported that an increase in cortisol secretion regarding the exercise status of individuals us subject to the volume of exercise, i.e. performing highly intense exercise can increase cortisol secretion [20].

Moreover, changes in cortisol levels during exercise have been explained by different mechanisms. Intense exercise, there is a two-fold increase in cortisol levels due to heightened secretion-excretion rate of cortisol [20]. In addition to plasma volume changes, variations in ambient temperature particularly increase by two degrees Celsius in core body temperature leading to catabolic stimulation and metabolic increased heat due to exercise, intensity and duration of activity can contribute to higher levels of cortisol [20]. In fact, cortisol levels rise in sports with an intensity of more than 60% of maximal aerobic power.
It should be noted that the stressful nature of the experiment due to psychological mechanisms could alter or increase the level of serum cortisol. With a long history of medical application, bilberry can act as an anti-hyperglycemia agent. Research shows that oral administration of bilberry can curtail hyperglycemia despite the presence of glucose injection at the same time. This effect is attributed to anthocyanosides myrtillus [21]. In addition, anthocyanosides in bilberry can enhance the integrity of collagen, stabilize the capillary permeability, and inhibit the accumulation of sorbitol, thus providing protection against the neurological complications of diabetes.

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