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Research Article

THE EFFECTS OF AEROBIC TRAINING AND ARBUTIN ON GLP1 AND GLP1R IN DIABETES RATS

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ABSTRACT
The aim of this study was showed effects of aerobic training (AT) and arbutin on GLP-1 and GLP-1R in diabetes rats. Experimental study was done on 42 male Wistar rats were randomly divided into six groups: control, diabetes, arbutin, arbutin/diabetes, diabetes/AT and diabetes/AT/arbutin. AT program consisted of running on a treadmill with 15-22 m/min intensity for 25-64 min, 5 times a week for 8 weeks. Arbutin supplemented was injected with 50 mg/kg intraperitoneally. An alloxan-induced rat (90 mg/kg) model of hyperglycaemia was used to evaluate the antihyperglycaemic. For determined the concentrations of Glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-1 receptor (GLP1R) by ELISA kits, serum samples taken from all groups after72 h cessation of AT and arbutin. Data were analyzed using ANOVA and dependent t-tests. After eight weeks, results showed reduces GLP-1 and GLP1R levels in diabetic group than in the control group ($P<0.05$). Administration of the arbutin (at doses of 500 mg/kg/day), AT and combination of AT/arbutin significantly increased the GLP-1 and GLP1R levels in alloxan-treated hyperglycaemic rats ($p=0.001$).This study demonstrated that a AT course along with the use of arbutin increase the GLP-1 and GLP1R levels in rats with alloxan-induced hyperglycaemia, also AT showed more effectiveness.

Keywords: *GLP-1, GLP-1R, Arbutin, Aerobic Training*

INTRUDUCTION
Type 2 diabetes mellitus (T2DM) is a lifelong disease and there is not yet cure and associated with both acquired and genetic risk factors (Malin *et al.,* 1998).
Centers for Disease Control and Prevention estimated that in 2000, 147 million people have diabetes, of which T2DM accounts for 90% to 95% of cases (Sheet *et al.,* 2009).The World Health Organization (WHO) estimates that the number of people with diabetes in range of 45-64 years old will be more than 140 million in developing countries and more than 30 million in developed countries in 2030 (Wild *et al.,* 2004).Various studies have shown that increasing prevalence of obesity and a decrease in physical activity are one of the important factors for diabetes (Mackelvie *et al.,* 2007; Saisho *et al.,* 2012; Arend *et al.,* 2006).Also, endocrine hormones such Glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-1 receptor (GLP1R) are the important factors that affected on body weight in T2DM patients (Simopoulos *et al.,* 1998).GLP-1 is a physiological mediator in regulatory and uptake pathways of glucose as well as gastric acid secretion and inhibits of glucagon secretion (Dharmalingam *et al.,* 2011; Giorda *et al.,* 2014; Meier *et al.,* 2004). Also, GLP-1 increases insulin secretion through mechanisms related to the control of voltage-dependent potassium channels that this hormone has been proposed as possible treatment T2DM (Mannucci *et al.,* 2000). In additions, the potential efficacy of physical activity is premised as fundamental defects implicated in the pathogenesis of T2DM that is associated with reduced muscle strength and metabolic control (Thomas *et al.,* 2006; Irvine *et al.,* 2009). Progressive resistance training (PRT) and aerobic training (AT) are considered a critical part for control of body weight, insulin sensitivity, glycemic control, blood pressure, lipid profile, fibrinolysis, endothelial function, and inflammatory defense systems (Ng *et al.,* 2011). That is considered as a preventative treatment for initiation and progression of T2DM (Arora *et al.,* 2009; Praet *et al.,* 2009). *Pyrus biossieriana Buhse* (locally known as wild pear) is a species of pear that is native to Iran (Khalilpour *et al.,* 2013). A resent herbal investigations show the α-amylase and α-glucosidase inhibitory properties of *Pyrus biossieriana Buhse* leaf extract (PbBLE) (Yousefi *et al.,* 2013). Arbutin is glycosylated hydroquinone that the largest

compound in the PbBLE that inhibits melanin formation by blocking tyrosinase (Rosa *et al.*, 2009; Gerich *et al.*, 2001).Also recent studies have been shown the antioxidant, antihyperglycaemic, and antihyperlipidemic effects of arbutin in the leukocyte-mediated inflammatory diseases (Hamed *et al.*, 2006).Increased insulin, glucose-induced muscle contraction with AT or PRT as a treatment tool for the patients with diabetes, for example Takii *et al.*, (1997) reported that arbutin due to antioxidant properties, lowers blood glucose (Takii *et al.*, 1997). Regarding to the importance of T2DM and potential role of physical activity and arbutin in the increase of insulin sensitivity, this study was performed to investigate the AT combined with the use of arbutin on concentrations of GLP-1 and GLP1R in induced T2DM rats.

MATERIALS AND METHODS

Plant Material and Extractions: The fresh leaves of *Pyrus boissieriana* buhse were obtained from their natural habitat in the north of Babol, Iran. After the plant was authenticated by the Mazandaran Department of Agricultural Sciences and Natural Resources, dried in the shade for 5 days and then the 500 g of plant extracted with methanol (63%) for 72 h at room temperature and evaporated to dryness under reduced pressure with a rotator evaporator(Azadbakht *et al.*, 2004; Shahaboddin *et al.*, 2011). The dried residue was dissolved in water and stored at -20 °C until further experimentation.

Study Animals: Experimental animals were all healthy adult male rats of the Wistar strain weighing 175.1±2.7 gr. Forty-two locally male rats were used in the study, purchased from Pastor Institute (Amol, Iran). All animals were housed individually under 12h light-dark cycle (lights on at 7:00 h) and controlled room temperature (24 ± 2 °C) with free access to cubes of standard rodent diet and tap water for at least 3-4 days before experimentation so that rodents could adapt themselves to the new environment and treadmill activity. All experiments were conducted after the approval from Local Animal Care Ethical Committee (ACEC).

Experimental Design: Animals were randomly divided into six groups of seven rats each. Group A (the control group) was fed 10 mL/kg/day of distiled water for 8 weeks, while group B (arbutin group) were gavaged 500 mg/kg/day of arbutin, from the first week for 6 weeks (5 days per week). Group C (diabetic group) only was diabetic for 8 weeks. Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (90 mg/kg). If blood glucose is more 250 mg/dL was considered as diabetes. Groups D and E were diabetic group Administered with AT, arbutin, respectively. Finally, group F (arbutin/diabetic/AT) was diabetic group recipient AT, arbutin together. After 72 h. the last AT session and 12-10 h. fasting, rats were anesthetized with intraperitoneally injection of combined ketamine (mg/kg50-30) and xylazine (mg/kg5-3), and taken immediately 5 ml blood from the left ventricle. Serum was obtained for GLP-1 and GLP1R analyses.

Exercise Training Protocol: Exercise training was performed on a motorized treadmill at moderate-to-low intensity (maximal running speed: 1.0 km/h), 5 days a week for 1 h/day (for 8 weeks), with 0% gradient. The animals were adapted to this procedure for 1 week before beginning the training with the speed of 5-8 m/min for 4-10 min, respectively, on consecutive days. AT program was performed on for 8 week with the speed of 15-22 m/min for 25-64 min, progressively. 1 min was added every day to rise exercise volume sedentary rats were placed on the stationary treadmill five times a week to create a similar environment **(**Winter *et al.*, 2007).According to the program, this condition corresponded to a moderate intensity of about 57–75% of maximal oxygen consumption (Ghanbari-Niaki *et al.*, 2007).

Plasma GLP-1 and GLP1R Assay: Concentrations of GLP-1 and GLP1R were determined by ELISA kits (Cusabio, china). The sensitivity of ELSA kit was 1.45 ng/ml and the correlation coefficient of the standard curve was 0.9995. The intra- and inter-assay CV was less than 8% (CV <8%).

Statistical Analysis: Kolmogorov–Smirnov test (K–S test) and Levene's test used to assess to evaluate the normality of the distribution and the equality of variances respectively. The results calculated statistically are expressed mean ± SD data were analyzed by one-way analysis of variance (ANOVA) using the statistical package for the social science (SPSS 21, spss ,Inc), with $p<0.05$ considered statistically significant, as assessed by Student's t-test with corrections for multiple comparisons to a single group (Dunnett's t-test) and between multiple groups (Tukey's tests).

RESULTS AND DISCUSSION

Findings

Table-1 shows weights of rats at the end of the eight weeks of AT experiment in the six groups. There was no significant difference in body weight between the research groups at the beginning of the study. After eight weeks AT, the animals' weight in arbutin/diabetic/AT group was less diabetic group, diabetic/arbutin group, diabetic/AT group and control group but was statistically insignificant.

Table 1: Weight changes of rats before and after 8 weeks aerobic training (ANOVA tests).

Groups Variable	control	arbutin	diabetic	diabetic+AT	diabetic+arbutin	diabetic+arbutin+AT
Initial weight (gr)	215±22	205±21	210±34	202±31	207±35	204±33
Final weight (gr)	235±25	232±23	247±37	237±35	240±27	232±28

All data expressed as a mean (±SD), AT: aerobic training

One-way analysis of variance on the GLP-1 and GLP1R levels indicated significant differences between six groups ($p<0.001$) (Table 2).

Table 2: Mean concentrations of GLP-1 and GLP1R in all groups

Groups Variable	control	arbutin	diabetic	diabetic+AT	diabetic+arbutin	diabetic+arbutin+AT
GLP-1 (ng/ml)	34.00±1.41	40.14±2.85	7.6±1.11	21.42±2.87	16.14±1.67	28.57±1.71
GLP1R (ng/ml)	2.91±0.20	3.28±0.30	1.44±0.20	2.37±0.19	1.82±0.16	2.58±0.24

All data expressed as a mean (±SD), AT: aerobic training

Table 3 show mean changes of GLP-1 levels at the end of the experiment in the Inter of all groups.

Table 3: Tukey test for GlP-1 Analysis between multiple groups

Groups	Arbutin	Control	Diabetic	Diabetic/Arbutin	Diabetic/AT	Diabetic/Arbutin/AT
Arbutin	-					
Control	M=6.142 p<0.001	-				
Diabetic	M=33.428 p<0.001	M=27.285 p<0.001	-			
Diabetic/Arbutin	M=24.000 p<0.001	M=17.857 p<0.001	M=9.428 p<0.001	-		
Diabetic/AT	M=18.714 p<0.001	M=12.571 p<0.001	M=14.714 p<0.001	M=5.285 P=0.002	-	
Diabetic/Arbutin/AT	M=11.571 p<0.001	M=5.428 P=0.002	M=21.857 p<0.001	M=12.428 p<0.001	M=7.142 p<0.001	-

All data expressed as a mean (±SD), M: Mean; AT: aerobic training

The baseline levels of the parameters were significantly different between the control and diabetic and AT groups (p<0.001). GLP-1 concentration levels significantly decreased at the end of the experiment in the study groups in comparison with the control group (p<0.001). On the other hand, GLP-1 concentration decreased about 5.5 folds from 27.3 µmol//L to 5.4 1µmol//L in the arbutin/diabetic/AT groups; however, the increase was significant statistically (p=0.002). Also, mean of GLP-1 levels had significant reduction in AT (P<0.001). There was a statistically significant in GLP-1 concentration in the all groups in comparison with the control group (p<0.001) (See the table 3).

Table 4 show mean changes of GLP1R levels at the end of the experiment in the Inter of all groups. GLP1R concentration levels significantly decreased at the end of the experiment in the study groups in comparison with the control group (p<0.001). In contrast to GLP-1, GLP1R concentration decreased about 0.33 folds in the arbutin/diabetic/AT groups; however, the increase was not significant statistically (P=0.208). Also GLP1R levels significantly don't changed at the end of the experiment in the arbutin groups in comparison with the control group (P=0.112). There was a statistically significant in GLP1R concentration in the diabetics and diabetic/AT groups in comparison with the control group (p<0.001) (See the Table 4).

Table 4: Tukey test for GLP1R Analysis between multiple groups

Groups	Arbutin	Control	Diabetic	Diabetic/Arbutin	Diabetic/AT	Diabetic/Arbutin/AT
Arbutin	-					
Control	M=0.371* P=0.112	-				
Diabetic	M=1.842 p<0.001	M=1.471 p<0.001	-			
Diabetic/Arbutin	M=1.457 p<0.001	M=1.085 p<0.001	M=0.385 P=0.089	-		
Diabetic/AT	M=0.914 p<0.001	M=0.542 P=0.004	M=0.928 p<0.001	M=0.542 P=0.004	-	
Diabetic/Arbutin/AT	M=0.7 p<0.001	M=0.328* P=0.208	M=1.142 p<0.001	M=0.757 p<0.001	M=0.214* P=0.66	-

*All data expressed as a mean (±SD), *p≥0.05 not significant; M: Mean; AT: aerobic training*

Discussion

This is the first study to date on the evaluation of AT and arbutin on glycemic control in induced type 2 diabetes rats. Our result revealed a significant difference between the aerobic training and arbutin groups (p≤0.05). And show the AT and arbutin complementary on blood glucose control in rats with hyperglycemia. Administration of the arbutin (at doses of 500 mg/kg/day), AT and combination of AT/arbutin significantly increased the GLP-1 and GLP1R levels in alloxan-treated hyperglycaemic rats (p=0.001). Preliminary results of the study showed that, glucose and insulin levels were significantly reduced in PRT (Arora *et al.*, 2009), AT (Praet *et al.*, 2009) and arbutin groups. Also many studies showed the relationship between physical activity and reduced glucose levels (Balducci *et al.*, 2012) . Recent studies have shown that during exercise, GLP-1 regulated glucose levels from expression of GLP1R in pancreas cell surface. Swanson *et al.*, (2010) show GLP-1 reduced 38% and 26% with concomitant use of arbutin and aerobic exercises, respectively. However, no observed significant difference between the increases in g GLP-1 between the three treatments groups (AT, arbutin and

AT/arbutin). Increased GLP-1 and GLP1R reduced the induced inflammation in T2DM mice that treated with AT, arbutin or AT/arbutin (Swanson *et al.,* 2010); our findings also confirm these results. But all study show the may be insufficient the AT period increased inflammation caused by T2DM.

Broom *et al.,* (2009) reported both aerobic exercise and resistance exercise are suppresses appetite significantly; But the mechanism is not completely characterized (Broom *et al.,* 2009). The results of Lee *et al.,* (2012) suggest that, swimming for four weeks, five days a week and an hour per session, decreases significantly glucose level (Lee *et al.,* 2012). On the other hand, increased levels of lactic acid in during AT is also possible mechanisms for appetite suppression. In other words, considering the conducted researches, the role of exercise in T2DM as increaser of sensitivity of cells to insulin, is well known while, there are little studies on the effect of physical activity in various intensities and the effect of AT on T2DM. Another significant finding in this paper is the effect of arbutin along with AT on the GLP-1 and GLP1R in the rats with hyperglycemia. Findings of this research approved with previous studies on the relationship of arbutin and glucose levels reduction (Yousefi *et al.,* 2013; Takii *et al.,* 1997; Azadbakht *et al.,* 2004).

Based on the other researcher's results, mechanism of the effect of exercise and with arbutin is unknown that proper explanation of the conflicting results of researches is not possible (Dharmalingam *et al.,* 2011; Praet *et al.,* 2009; Khalilpour *et al.,* 2013). However, the certain topic is that, arbutin decreases the blood glucose lonely due to antioxidant property; hence, it can be used as a contributing factor to control T2DM (Dharmalingam *et al.,* 2011; Mannucci *et al.,* 2000; Yousefi *et al.,* 2013). According to the results of this paper, after eight weeks AT a significant reduction was observed in GLP-1 and GLP1R levels. Also it was observed that, arbutin with AT led to lower GLP-1 and GLP1R levels n T2DM in rats.

Conclusion

In conclusion, considering the significant reduction of GLP-1 and GLP1R levels, we recommend the use of AT/arbutin as an alternative strategy in reduction or improvement of insulin sensitivity and complications in T2DM patients. However, further studies are still needed to provide more evidence on the effectiveness of AT/arbutin.

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