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Original Research

Effect of Biochanin A on Serum Nesfatin-1 Level in STZ Induced Type 1 Diabetic Rat

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Abstract

Bioflavonoids such as biochanin A (BCA), exhibit insulin mimetic or secretagogues activities and are considered as hypoglycemic compounds. Nesfatin-1 is secreted from the hypothalamic nuclei and recognized as a regulatory peptide, which can increase insulin sensitivity and affect glucose metabolism. In this study, effects of BCA, on serum nesfatin-1 level were examined in rats with streptozotocin (STZ)-induced diabetes. We randomly divided 30 male Wistar rats into 2 control (6 rats per group) and 3 diabetic groups. Type I diabetes was induced using STZ (55 mg/kg bw) injection. Group 1 received 0.5% DMSO; while group 2 received 10 mg/kg body weight of BCA; group 3 (diabetic controls) received 0.5% DMSO; group 4, 10 mg/kg of BCA; and group 5, 15 mg/kg of BCA. The levels of serum insulin, nesfatin-1, and fasting blood glucose (FBG) were determined after 42 days. The serum insulin level increased; while FBG level significantly decreased in the BCA treatment groups. Both treatment groups had increased nesfatin-1 levels in comparison with the control groups; however, the difference was only significant in group 5 (p value < 0.05). Considering insulin and nesfatin-1 induction, BCA has potential hypoglycemic effects. Oral administration of 15 mg/kg BW of BCA showed greater efficacy than 10 mg/kg bw of BCA.

Keywords: Nesfatin-1; Biochanin A; Diabetes; Rat

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Introduction

Diabetes mellitus is described as a chronic disease, associated with metabolic disorders and chronic hyperglycemia. Lack of insulin secretion, insulin function, or both causes various disorders in glucose homeostasis and carbohydrate metabolism [1]. In addition to excessive energy storage, several proteins are produced and released from adipose tissues, such as adipocytokines, which play important roles in regulating metabolic homeostasis and pathogenesis of diabetes mellitus [2].

Nesfatin-1 is an anorectic protein with 82 amino acids, originating from the precursor molecule on NUCB2 gene [3]. It has been identified in several regions of hypothalamus tissues, such as gastric mucosa, adipocytes, pancreatic beta cells, and pituitary autonomic nuclei [4]. According to the literature, body weight reduces by intracerebroventricular (ICV) nesfatin-1; therefore, nesfatin-1 is known as a satiety molecule [3].

Peripheral nesfatin-1 is responsible for controlling glucose homeostasis [5]. Nesfatin-1 triggers the secretion of glucose-induced insulin by increasing Ca+2 ion influx [6]. It has been demonstrated that in some tissues (e.g., adipose tissues and skeletal muscles), insulin sensitivity and secretion increase by nesfatin-1 through changing GLUT-4 translocation and AKY phosphorylation [7].

Although a variety of oral hypoglycemic medicines have been developed, they are quite costly in developing countries has and have negative effects and limited efficacy. The low cost of phytochemicals and their limited side effects can promote the treatment of various disorders such as diabetes [8]. Bioflavonoids, as well-recognized phenolic compounds, show strong antioxidant activities. Although these phenolic compounds are not conventional hydrogen-donating antioxidants, they can trigger modulatory activities via different signaling pathways [9].

Presence of biochanin A (BCA) has been reported in soy, red clover, peanuts, chickpea, and alfalfa sprouts [3]. It is known as an antidiabetic agent with various biological benefits, such as anti-inflammatory and anti-neoplastic characteristics [10]. Therefore, the effects of BCA on serum fasting blood glucose (FBG) and nesfatin-1 were examined in the present study on diabetic rats.

Methods

Chemicals and Reagents

Sigma Aldrich Co. provided STZ, BCA, and dimethyl sulfoxide (DMSO), while E-Merck supplied the rest of analytical-grade chemicals. A commercial kit (Pars Azmon, Iran) and a spectrophotometer (Jenway 6505, European Union) were used to measure the FBG serum level according to glucose oxidase method. An ELISA kit (Bioassay Technology Laboratory, Shanghai, China) was also used for insulin measurement; while nesfatin-1 level was determined with the ELISA kit (Cusabio, China), using an ELISA plate reader (Biotek ELX800TM) based on the manufacturer's instructions.

Animals

In this experimental study, the animal house of Tehran University of Medical Sciences provided

the male Wistar rats (n = 30; 200-220 g). The animals were kept under standard temperature and humidity and had access to food and water. The ethics committee of Arak University of Medical Sciences approved this study (IR.ARAKMU.REC.1394.227). All efforts were made to reduce animal suffering. On the first and last days of oral administration, the rats' body weight was measured (Sartorius, Germany).

Animal groups and diabetes induction

We included 2 control groups in this study (6 rats per group): normal control (group 1), 0.5% DMSO; and BCA control (group 2), 10 mg/kg bw of BCA. For inducing type I diabetes in other rats, STZ (i.p.; 55 mg/kg bw) was injected. Induction of diabetes was confirmed by determining the increase in FBG level after 72 hours. Rats with FBG above 250 mg/dl were confirmed as diabetic and randomly divided in 3 groups: group 3 (0.5% DMSO; diabetic controls); group 4 (10 mg/kg BCA); and group 5 (15 mg/kg BCA) for 42 days. These concentrations of BCA were selected based on our previous researches. After that, the animals were anesthetized using Ketamine (75 mg/kg b.w) and Xylazine (10 mg/ Kg b.w) intraperitoneally. Blood sample was collected by cardiac puncture and serum was separated immediately. FBG, insulin and nesfatin-1 serum levels were evaluated as mentioned above.

Table 1: Examined Groups

Treatment	Group number	
0.5 % DMSO	1	Non diabetic Control Group
10 mg/kg BCA	2	
0.5 % DMSO	3	STZ induced Diabetic Group
10 mg/kg BCA	4	
15 mg/kg BCA	5	

Statistical analysis

The data (from 3 replicates) are presented as mean±SD (6 rats per group). Stata version 13 was used for Statistical analysis. Shapiro-Wilk test was applied to test the normality assumption. Moreover, to evaluate differences between variables, one-way ANOVA was performed. For comparison of data, Tukey's test was used. The significance level was $P < 0.05$.

Results

The effects of BCA on the serum FBG level are presented in Figure 1. FBG was significantly increased among diabetic controls versus the non-diabetic controls ($P < 0.05$). As the findings revealed, 10 and 15 mg/kg bw of oral BCA significantly reduced FBG level in diabetic rats ($P < 0.05$); however, BCA doses were not significantly different.

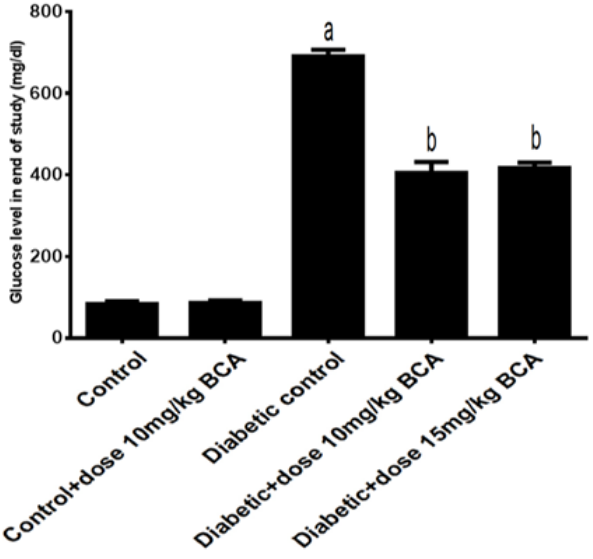

Figure1. Blood glucose in the control and diabetic groups treated with BCA for 6 weeks. Data are presented as mean ± SD for 6 rats per group: a: $P < 0.05$, versus normal rats; b: $P < 0.05$ versus diabetic rats.

Figure 2 presents the effects of BCA on nesfatin-1 changes in normal and diabetic groups. The level of nesfatin-1 was increased in diabetic rats in comparison to the controls; nonetheless, no significant difference was found. In diabetic rats, the level of nesfatin-1 was significantly increased by oral BCA administration at 15 mg/kg bw ($P < 0.05$).

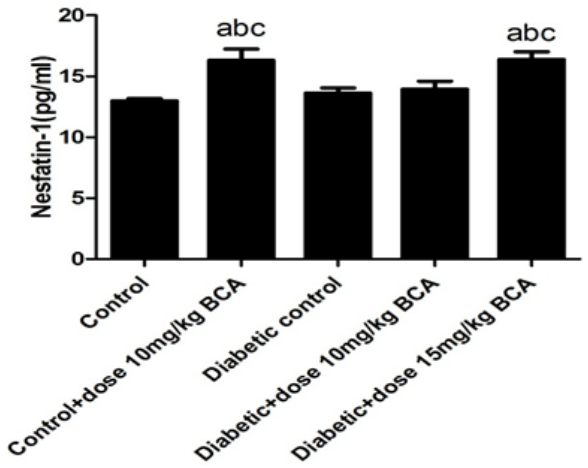

Figure 2. Serum nesfatin-1 in diabetic and control rats receiving BCA. Data are presented as mean ± SD for each group, a: $P < 0.05$ versus the controls; b: $P < 0.05$ versus the diabetic controls, c: $P < 0.05$ versus diabetic rats receiving 10 mg/kg of BCA.

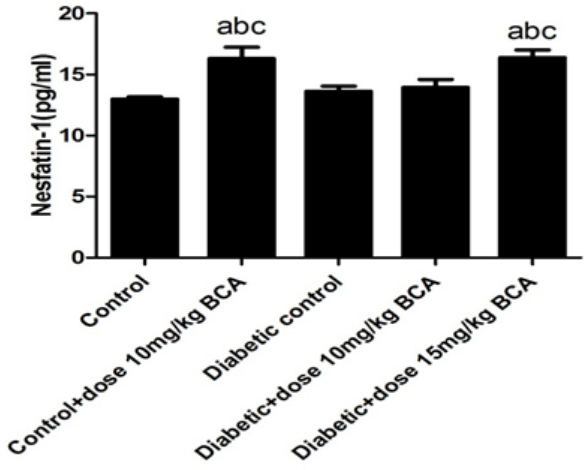

Figure 3. Serum insulin in the control and diabetic groups treated with BCA for 6 weeks: a, $P < 0.05$ versus the controls; b, $P < 0.05$ versus the diabetic controls.

Figure 3 presents the effects of oral BCA on serum insulin level. Based on the findings, the serum level of insulin was significantly reduced in diabetic rats. Insulin secretion was significantly increased following BCA treatment for 42 days in all treated rats.

A significant reduction was observed in body weight in the diabetic control group. Both doses of BCA could improve body weight. Based on the findings, 15 mg/kg BW of BCA showed greater efficacy than 10 mg/kg bw of BCA in comparison with untreated rats; nonetheless, no significant difference was found (data not shown).

Discussion

BCA oral administration showed significant effects on serum glucose restoration. Harini et al. and Azizi et al. indicated that oral BCA administration could reduce FBG serum level in diabetic rats [12,13]. BCA was associated with circulating insulin enhancement and induced protective effects on pancreatic beta cells, as well as insulin secretion from the remaining beta cells [13]. Wang et al. Introduced BCA as a potent PPARγ agonist, which can mediate anti-diabetic actions [14]. The diabetic rats significantly lost weight in comparison with the control rats which can be attributed to tissue protein breakdown in diabetic rats and in glucose metabolism [15].

In BCA-treated rats, the level of circulating insulin increased, which might contribute to the decrement of hyperglycemia and improvement of body weight. Nesfatin-1 is able to regulate food intake and contribute to glucose metabolism [5,16]. It possibly symbolizes a new factor of insulin contributors [4].

Our study revealed that nesfatin-1 concentration is increased in type I diabetic rats. This finding is consistent with a study by Li *et al.,* which showed a slightly higher plasma nesfatin-1 level in T1DM patients in comparison with the controls; nonetheless, the difference was insignificant [17]. Recent studies indicated that hyperglycemia in hypothalamic neurons activates the expression of nesfatin-1, and pancreatic cells improve with high serum levels of glucose [18]. In another study, increase of Ca+2 influx in pancreatic β-cells was improved by insulin secretion via L-type calcium channel activation [6]. Our findings showed that nesfatin-1 was increased in the diabetic group, and 15 mg/kg bw of BCA could significantly increase its level. Oh et al. reported the potential of PPARγ ligands in the pre-adipocyte cell line in improving the expression of NUCB2 gene [3]. Moreover, Yamada et al. showed that this stimulatory activity might be attributed to stabilization of NUCB2 mRNA, not activation of transcriptional genes in the ERK1/2 pathway [19]. Overall, BCA is a potent PPARγ ligand [14,20]. Literatures show the crucial role of PPARγ ligands on nesfatin-1 stabilization and significant association between serum nesfatin-1 and PPARγ in obese subjects [19,21].

We suggest that administration of BCA as a PPARγ agonist can elevate the expression of nesfatin-1. It can be concluded that BCA administration leads to glucose homeostasis improvement and increases the nesfatin-1 level. The limitation of this study is the lack of confirmatory test methods for nesfatin-1 assay. Also, it should be mentioned that the increase in nesfatin-1 level was more affected by BCA administration rather than diabetes induction since the level of this marker was significantly elevated even in non-diabetic animals treated with BCA.

Future studies are needed to further clarify the role of BCA in diabetes management via nsfatin-1.

Conflicts of Interest

None.

Acknowledgments

None.

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