Original Article

Effects of Biochanin A on Resistin, Adiponectin and Some Stress Oxidative Markers in Normal and STZ- Induced Diabetic Rats

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Received: 21 April, 2018; Accepted: 23 July, 2018

Abstract

Background: Elevated serum level of adiponectin and insulin as well as decreased serum resistin level can improve glucose metabolism. Biochanin A (BCA) is a flavonoid of Soybean that shows antioxidant properties. This study was aimed to examine the effect of BCA on fasting blood sugar (FBS), oxidative stress and serum levels of adiponectin, resistin and insulin in rats with type 1 diabetes. **Materials and Methods:** The rats were randomly divided into five groups (n=6). BCA was administered orally in doses of 10 and 15 mg/kg of body weight. Insulin, resistin and adiponectin were measured using ELISA kits. The activity of Gamma-Glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the levels of Glutathione (GSH) were examined. **Results:** The results showed that BCA treatment significantly reduced the FBS level in diabetic rats ($p<0.05$). Serum insulin was significantly increased in the BCA treated diabetic rats ($p<0.05$). Moreover, GGT activity and GSH was significantly increased in treated rats ($p<0.05$). Our findings revealed that the administration of BCA significantly increased the serum adiponectin ($p<0.05$). Additionally, serum resistin levels were remarkably decreased in treated rats ($p<0.05$). **Conclusion:** Taken together, BCA represents a natural phytoestrogen that has an important role in improvement of glucose metabolism by regulating of adipokines secretion. Our findings slso revealed the beneficial effects of BCA against oxidative stress in diabetes.

Keywords: Adiponectin, Biochanin A, Oxidative stress, Resistin, Type 1 diabetes.

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Please cite this article as: Salehi E, Ghasemi H, Morovati A, Sadri H. Effects of Biochanin A on Resistin, Adiponectin and Some Stress Oxidative Markers in Normal and STZ- Induced Diabetic Rats. Arch Med Lab Sci. 2018;4(2):9-16.

Introduction

Type 1 diabetes is an autoimmune disorder caused by a destruction of pancreatic beta cells by immune cells (1). Pancreatic β-cell destruction usually leads to absolute insulin deficiency and impaired glucose homeostasis (2).

Diabetes is one of most challengeable health issue nowadays (1). In 2017, almost 370 thousand Americans were diagnosed with type 1 diabetes, which is about 10% of all people with diabetes (2). Taking insulin is the best treatment for type 1 diabetics; however, it is difficult to maintain the glucose level close to normal to delay or prevent complications (3). Cardiovascular disease, neuropathy, nephropathy and retinopathy are the main complications of diabetes that directly or indirectly are associated with a high blood glucose level (4). Hyperglycemic patients are affected by microvascular and macrovascular complications through different pathways including: 1) increase the activity of polyol pathway, 2) protein kinase C activation, 3) advanced glycation products formation

and 4) increase hexosamine pathway flux (5). Although, the insulin deficiency is the primary metabolic defect of type 1 diabetes, a number of studies have reported that various levels of insulin resistance also exist in type 1 diabetic patients (6, 7). Adipocytes are endocrine cells in adipose tissue that secret different adipokines that play important roles in glycemic control (8, 9).

Adiponectin is one of the important adipokines secreted by adipocytes that has hypoglycemic properties and prevents insulin resistance. Several studies have shown that in obesity and insulin resistance, adiponectin level reduced (10). Resistin is another adipokine that belongs to the family of cysteine-rich proteins that secretes from adipocytes and reduces the insulin sensitivity (11). Furthermore, various studies suggested systemic oxidative stress could be induced by obesity, consequently, causing dysregulation of adipokines and metabolic syndrome and diabetes development (12).

Increased oxidative stress is an extensively endorsed participant in the progression and development of diabetes and its complications. ROS produced by the mentioned pathways causes oxidative stress and lack of endogenous antioxidant agent lead to cell damage (5, 13). GSH as an endogenous defense against oxidative stress, is made from three amino acids include cysteine, glutamic acid and glycine. Its antioxidant action is through oxidation and reduction in the thiol group in its structure. GGT enzyme, which directly involves in the metabolism of GSH, is found in the cell membrane and regulates the GSH level (14).

Traditional medicine has been used to treat different diseases for many years worldwide. Using herbal medicine to reduce blood glucose and improvement of hyperlipidemia has been examined for a long time by diabetic patients (15). Flavonoids are the most abundant part of the plants that has been identified; using this plant ingredient due to its estrogenic properties leads to hypolipidemia and can improve diabetes curing. Additionally, antioxidant property of these derivatives is well proven. BCA is a methylated form of Soy

isoflavone which is found in red clover, cabbage, and alfalfa. BCA shows different pharmacological properties such as anticancer, antidiabetic, anti-inflammatory, antiallergic, hepatoprotective, and neuroprotective (16). Additionally, previous studies revealed that BCA is a potent radical scavenger (17). Hence, regarding to the antioxidant properties of BCA, we examined the effect of BCA on the secreted adipokines, insulin levels and insulin resistance index or HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) in type 1 diabetic rats.

Methods

Animals. Forty male Wistar rats (body weight 200-250 g) were purchased and maintained at a controlled temperature (22±4 °C) and 12-hour light/dark period conditions. Animals were fed with standard water and chow during the study. The ethics committee of Arak University of Medical Sciences approved the protocol of this animal study (project number 93-186-28).

Diabetes Induction. To induce type 1 diabetes Streptozotocin (STZ) powder was dissolved in citrate buffer (pH=4.5) and the injection was performed intraperitoneally (55 mg/kg body weight). Seventy two hours after injection, to confirm the development of diabetes, fasting blood samples were taken from the rats and blood sugar was measured using a glucometer. The animals having FBG 250 mg/dl or higher were considered diabetic (18). Blood samples were taken from the tail vein of animals (19).

Examination Procedures. Animals were divided randomly into 5 groups (n=6). BCA (Sigma) was dissolved in 5% dimethyl sulfoxide (DMSO) and administrated orally (10 and 15 mg/kg bw) using gavage syringe for 42 days.

The groups were as follows; Group 1: Healthy control (5% DMSO), Group 2: Healthy control + BCA (10 mg/kg), Group 3: Diabetic control (5% DMSO), Group 4: Diabetic + BCA (10 mg/kg), Group 5: Diabetic + BCA (15 mg/kg).

After 42 days treatment and following 12 hours fasting, the animals were anesthetized using intraperitoneal injection of ketamine and xylazine. Blood samples were taken from a vein in their hearts and then serum was separated and frozen at -20 °C. The body weight of the rats was measured before and

after treatment.

Biochemical Analysis. Serum insulin was measured using ELISA kit (Bioassay technology laboratory Shanghai, China).

Insulin resistance index HOMA-IR was calculated as follows (20):

$$\text{Insulin } (\mu U/ml) \times \text{glucose (mmol/L)}/22.5$$

Resistin and adiponectin were measured using ELISA kits purchased from Shanghai Crystal D Biotech companies (China). To compare metabolic condition in study groups, serum level of glucose was measured using an enzymatic method.

The activity of AST, ALT and GGT enzymes were measured using commercial colorimetric kits (Pars Azmun, Tehran, Iran) and a spectrophotometer (JENWAY 6505, Europe Union). Serum GSH was measured using colorimetric kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical Analysis. Statistical analysis of obtained data was performed using SPSS software, version 16. Firstly, the data distribution in each group was assessed for normality and then the statistical comparison was carried out using ANOVA test and post hoc Tukey test. In all statistical analyzes p-value less than 0.05 was considered significant.

Results

The Effect of BCA on FBS. In this study, fasting blood glucose (FBG) was measured at the beginning and end of the study, our results showed that FBG levels significantly increased in diabetic rats compared to healthy control rats ($p<0.05$). A comparison of blood glucose levels in treated groups with doses of 10 and 15 mg/kg BCA and the diabetic control group showed significantly lower level ($p=0.001$) in treated groups. However, comparing the two treated groups (10 & 15 mg/kg BCA), no significant differences in FBG after 6 weeks of treatment was observed (Fig. 1). Moreover, our results revealed that diabetic rat's body weight (BW) was decreased significantly. Oral administration of BCA at two different doses (10 & 15 mg/kg BCA) improved BW; however, the difference in gained weight was not statistically remarkable.

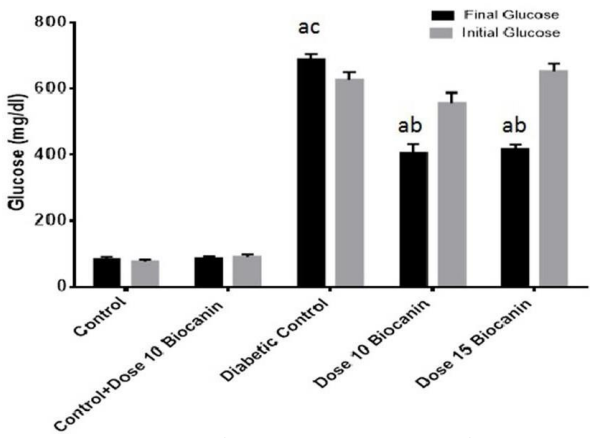

Figure 1. Comparison of FBSat the start and end of the study: a: compare with beginning in same group ($p<0.05$), b: compare with diabetic control ($p<0.05$), c: compare with healthy control ($p<0.05$).

The Effect of BCA on Serum Level of Adiponectin. As shown in Figure 2, adiponectin level in the diabetic group compared with the healthy control group was significantly lower ($p=0.001$). In addition, analysis of data showed a significant difference in adiponectin level between treated groups (doses 10 and 15 mg/kg) and the diabetic control group. Adiponectin level in two treated groups significantly increased compared to the diabetic control group ($p=0.001$). However, there was no significant difference in adiponectin level between two treated groups.

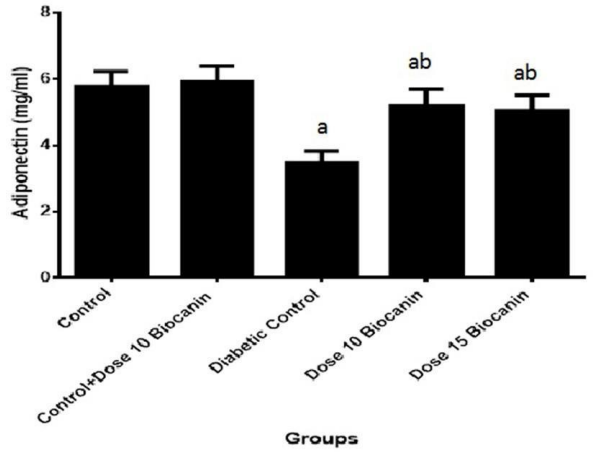

Figure 2. Comparison of serum adiponectin level in study groups a: compare with healthy control ($p<0.05$), b: compare with diabetic control ($p<0.05$).

The Effect of BCA on Serum Level of Resistin. As Fig. 3 shows, resistin levels in the diabetic control group was significantly increased compared to the healthy control group (p=0.001), on the other hand, the results showed that resistin was significantly lower in treated rats (10 & 15 mg/kg BCA) than in diabetic control rats (p=0.001). There was no significant difference in resistin concentrations between two treated groups (10 & 15 mg/kg BCA).

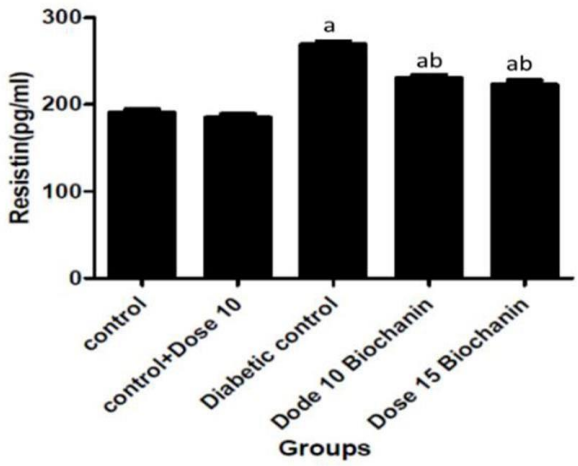

Figure 3. Comparison of serum resistin level in study groups. Data analysis showed that resistin level increased in diabetic rats (p=0.001), a: compare with control (p<0.05), b: compare with diabetic control (p<0.05).

The Effect of BCA on Insulin Level and HOMA-IR. In Table 1, results of insulin and insulin resistance index (HOMA-IR) are shown; as these data show, the concentration of insulin in the diabetic group was significantly lower than that of the healthy control group (p=0.001). Also, analysis showed that insulin levels were significantly increased in two treated groups (doses 10 and 15 mg/kg). No difference was observed in the insulin level between the two treatment groups (doses 10 and 15 mg/kg). Clearly, in the diabetic control group, HOMA-IR increased significantly compared to the healthy control group (p=0.001). Moreover, HOMA-IR remarkably decreased in treated groups (doses 10 and 15 mg/kg) when compared with diabetic control (p<0.05). This index in the treated group with BCA doses of 10 was less than that of the treated group with the higher dose of BCA; however, the difference between the treatment groups was not statistically significant.

The Effect of BCA on GGT Enzyme Activity. As shown in Table 1, analysis of the results demonstrated that the activity of GGT enzyme remarkably elevated in the diabetic control group compared to the healthy control group (p=0.001). Also, a significant decrease in GGT was observed in the two treated groups (10 & 15 mg/kg BCA) compared with the diabetic control group (p<0.05). However, no significant differences were observed in GGT activity between two groups that treated with different doses of BCA.

The effect of BCA on GSH level. There was a significant difference in the mean of the GSH level between the study groups (p=0.001). GSH in the diabetic control group significantly reduced in comparison with the healthy control group (p=0.001). In both treatment groups, there was a significant increase in the concentration of GSH level compared to the diabetic control group (p=0.001) (Table 1).

Table1. Effect Biochanin A on serum insulin level, HOMA-IR index, GGT activity and GSH level in healthy, type 1 diabetic and treated diabetic groups.

Groups	Healthy control	Healthy control+BCA 10 mg/kg	Diabetic control	Diabetic+BCA 10 mg/kg	Diabetic+BCA 15 mg/kg
HOMA-IR index	2.78 ± 0.09	2.91 ± 0.13a	12.56 ± 1.99a	10.71 ± 0.98ab	11.75 ± 0.45b
Insulin(mIU/L)	4.5 ± 0.25	3.6 ± 0.55	2.45 ± 0.45a	4.58 ± 0.49ab	4.0 ± 0.52 b
GGT (U/L)	2.16 ± 0.40	2.33 ± 0.5	30 ± 4.24 a	25.33 ± 1.36ab	24.85 ± 3.89 ab
GSH (µmol/ml)	0.803 ± 0.031	0.760 ± 0.024	0.694 ± 0.016a	0.739 ± 0.039 ab	0.746 ± 0.021ab

Results are presented as mean ± SD. BCA, Biochanin A; GGT, Gamma-Glutamyl transferase; GSH, Glutathione

Discussion

In this study, antidiabetic properties of BCA were examined in healthy and STZ- induced diabetic rats. STZ, a drug with various functions, due to its toxicity on β cells islets of Langerhans of the pancreas, interferes with the oxidative mechanisms of cells leads to diabetes mellitus in animal models (21).

Diabetes mellitus as an important metabolic disorder was characterized by insulin secretion deficiency or defect in insulin function which results in hyperglycemia. As well as the chronic hyperglycemia caused by insulin deficiency is linked to long-term dysfunction, damage of various organs.

Type 1 diabetes mainly caused by insulin secretion deficiency is associated with damage to β cells which eventually results in weight loss. In fact, inability to use glucose in insulin sensitive cells and protein synthesis is the main weight loss reason (22). Our finding has demonstrated that type 1 diabetes caused weight loss in diabetes induced rats, however, in this study results revealed that BCA treatment caused weight gain over the treatment period. Although, this weight increases did not reach to the healthy controls weight; there was a remarkable difference in BW between treated groups and healthy controls (Fig. 2). According to recent studies reports, BCA improves the function and secretion of insulin, which it suggested that the BCA consumption leads to gained weight associated by improving glucose metabolism. Previous studies reported that type 1 diabetes leads to weight loss in result of proteolytic degradation and loss of ATP and energy as well as lack of protein synthesis (23).

The results of present study showed that BCA treatment reduced serum glucose in type 1 induced rats; BCA antidiabetic properties in type 1 diabetes associated with increasing the insulin production in remnant β cells. In accordance with present study results, Harni and colleague showed that BCA consumption reduces blood glucose levels; they suggested that the glucose level decline could be caused by insulin secretion increment (21). On the other hand, Insulin assay in our study showed that BCA consumption increased insulin secretion. Moreover, HOMA-IR was higher in the diabetic group compared to other groups that received BCA; these results confirm the BCA effects on insulin secretion. Various studies have reported that flavonoids' anti-diabetic properties could improve insulin resistance; also it increases insulin secretion from pancreatic beta cells (24, 25). Flavonoids' anti-diabetic effects carried out based on different mechanisms in various cells especially in pancreatic beta cells, these mechanisms mainly includes: increasing insulin secretion to reduce hyperglycemia by improving glucose metabolism in the hepatocytes, increasing glucose uptake in adipose tissue and skeletal muscle, also reduced β-cell apoptosis and increased the cell proliferation are the main mechanisms which are improved by Flavonoids (15).

Elevated adiponectin secretion is one of the important factors that lead to enhanced insulin function in skeletal muscle cells. Adiponectin as an adipose tissue secreted hormone could improves insulin function in insulin dependent cells such as skeletal muscle cells. In this way, adiponectin binds to its specific receptor and activates the AMPK and PPAR-α and then resulting in enriched insulin function. Therefore, adiponectin reducing has a negative effect on insulin function (26, 27). Our finding showed that BCA administration increased the adiponectin secretion. Resent findings revealed that BCA and Thiazolidinediones could act as a PPAR-γ agonists and raise the adiponectin production, then improve the insulin function (28). In accordance to present study results Ghadimi et al. showed that intraperitoneal and oral administration of BCA in type 2 diabetic rats increased insulin secretion and decreased insulin resistance (29).

Resistin is also another adipokine derived from adipocytes that has an important role in pathophysiology of diabetes. Our study showed that resistin increased in diabetic group compared to the control group; these changes are parallel to the results of glucose and HOMA-IR and BW. Resistin levels also significantly decreased in the BCA treated groups compared with diabetic control group. In accordance with present study results, recent study

which is carried out by Asterholm and colleague demonstrated that increased resistin level could play an important role in insulin resistance (30). Resistin as an important adipocyte derived chemokine could acts as an inflammatory agent and leads to insulin resistance and also it could improves the risk of diabetes and cardiovascular diseases (31). It has been reported that in diabetic patients' resistin level is higher; in these diabetic patients resistin acts as an insulin antagonist which leads to oxidative stress induced by glucose toxicity (32). From this view, high glucose associated with type 1 and type 2 diabetes induced oxidative stress-damage (33).

Our results indicated that the diabetic group had higher GGT activity than healthy control. Also results showed that GSH remarkably decreased in diabetic rats. GSH and GGT considered as two oxidative stress markers mainly altered in diabetic patients. GSH as an important cellular defense against oxidative stress basically regulated by GGT enzyme (34). Decreased GSH level with a concurrent increase in GGT activity in diabetic rats strongly suggest the diabetes induced oxidative stress. On the other hand, Consumption of BCA as an antioxidant compound improves the antioxidant condition in treated rats. It has been reported that, BCA consumption and its antioxidant properties are closely paralleled by the alteration in GGT activity and GSH level. Ravuri et al. showed that GGT gene expression increases in cells resulting in mitochondrial oxidative stress (35). GSH as another oxidative stress marker that possesses an important cellular defense against oxidative stress is used to prevent the lipid peroxidation and oxidative stress in diabetic patients and decrease in GSH level is a risk factor for diabetes (36). In accordance with our study, the results of Ansely et al. showed that hyperglycemia related to diabetes is significantly associated with decreased GSH level and depilation of GSH in diabetic patients favors damage caused by high glucose (37, 38).

Additionally, in present study, we found that liver damage caused by toxic agents such as STZ (39) is prevented by using natural antioxidants component such as BCA. In this study, the rats with type 1 diabetes had much more ALT and AST activities compared to healthy rats. While in diabetic rats received BCA, the activity of the liver enzyme decreased significantly which indicates a protective effect of BCA against induced liver damage. Our previous results showed that STZ could induce higher ALT and AST activity in type 1 diabetic rats in comparison with normal rates, and using flavonoids could exert liver protective effects in STZ induced type 1 diabetes (20).

CONCLUSION

It can be concluded that BCA as a natural antioxidant agent could have beneficial effect on insulin secretion, insulin sensitivity and antioxidant status in type 1 diabetic rats. Furthermore, the oral administration of BCA can control the secretion of adipocytes derived hormones such as adiponectin and resistin which have a vital role on the initiation and development insulin resistance.

ACKNOWLEDGMENT

This study was supported by grants from Arak University of Medical Sciences.

Conflict of Interest

The authors declare no competing interests.

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