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The effect of Biochanin A on the expression of Adiponectin in adipose tissue of Streptozotocin-Nicotinamide induced diabetic rats

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ABSTRACT

Increased plasma level of adiponectin can improve insulin resistance in skeletal muscle cells. Activation of PPAR γ transcription factor can increase the expression of adiponectin gene and adiponectin level. Biochanin A, a natural compound, is a PPAR γ agonist. The aim of this study was to investigate the effect of Biochanin A administration on adiponectin expression, insulin resistance and control of type 2 diabetes mellitus. Adult male Wistar rats were used in this study. Induction of type 2 diabetes mellitus was performed by single intraperitoneal injection of Streptozotocin-Nicotinamide. Animals were randomly divided into 8 groups and Biochanin A was administered for a month at 4 different doses: 1 and 5 mg/kg IP, 10 and 20 mg/kg orally. Some biochemical parameters such as fasting blood glucose, plasma insulin and HOMA index were measured. Adiponectin level in adipose tissue was examined by western blotting analysis. Results of the present study demonstrated that Biochanin A consumption at doses of 5mg/kg IP, 10 and 20mg/kg orally decreased the fasting blood glucose level, increased adiponectin level in adipose tissue and improved insulin resistance significantly. Based on findings of present study, it can be concluded that increased adiponectin level in adipose tissue accompanies with improved insulin resistance.

Keywords: Insulin resistance, Type 2 diabetes mellitus, Adiponectin, Adipose tissue, Isoflavonoid, Biochanin A

INTRODUCTION

Type 2 diabetes mellitus is defined as a chronic metabolic disorder characterized by increased blood glucose level, this often results from insulin resistance. Type 2 diabetes mellitus strongly associates with sedentary lifestyle and obesity. Obesity can lead to insulin resistance; a disorder in which skeletal muscle cells and adipocytes cannot uptake glucose via glucose transporter 4 [Glut 4][6,12, 22,24].

Hyperglycemia can accompany with complications such as cardiovascular diseases, nephropathies and neuropathies. According to epidemiological studies, world prevalence of type 2 diabetes mellitus was 6.4% in 2010; it will increase to 7.7% in 2030; that can affect 439 million people all over the world[24,29].

Nowadays there are several anti-diabetic drugs with different mechanisms, but their long-term use can accompany with adverse side effects such as hypoglycemia, hepatotoxicity and nephrotoxicity. Because of the diabetic complications and the side effects of prolonged use of anti-diabetic drugs, management of diabetic patients is still a challenge in developed countries.

Recently, plants and natural products such as flavonoids have opened new avenues in the control of type 2 diabetes mellitus. Flavonoids are a class of plant secondary metabolites. They can be classified into: flavonoids, isoflavonoids and neoflavonoids. BiochaninA [BCA] is an O-methylated isoflavone. BCA can be found in red clover, soy and some legumes. In several studies, the anti-diabetic effect of BCA was approved, but its function and mechanism is still controversial. Probable anti-diabetic mechanisms that were indicated in different studies are stimulation of insulin secretion from beta pancreatic cells, insulin mimetics effect, increasing of glucose uptake in peripheral tissues and regulating the key enzymes in carbohydrate metabolism. Some researchers believe that BCA is a peroxisome proliferator-activated receptor γ [PPAR γ] agonist. PPAR γ is a ligand inducible transcription factor, mainly presents in adipocytes, colon and macrophages and regulates glucose homeostasis[2,11,21,27].

The activated genes by PPAR γ , stimulate glucose uptake by adipocytes and skeletal muscle cells. One of the genes, that has PPAR γ - responsive element [PPRE] in its promoter, is adiponectin gene or adipocyte complement related protein of 30kDa [Acrp30]. Adiponectin is an adipocyte-derived hormone that has an important role in glucose homeostasis and in several studies is known as an anti-diabetic agent that can improve insulin resistance. Its concentration in plasma is approximately 5-20 μ g/ml. Adiponectin receptors are mainly classified into adiponectin receptor 1 [Adipo R1] and adiponectin receptor 2 [Adipo R2]. Adipo R1 exists on the surface of skeletal muscle cells and Adipo R2 exists only on the surface of hepatocytes[3,5,17]. PPAR γ agonists such as BCA, can activate PPAR γ as a transcription factor and activation of PPAR γ will lead to increase in adiponectin gene expression and adiponectin level eventually [3,11,20].

This study was designed to investigate the effect of BCA administration on adiponectin expression in adipocytes of Streptozotocin-Nicotinamide [STZ-NCD] induced diabetic rats. Additionally, the effect of BCA on body weight [BW], fasting blood glucose [FBG] and plasma insulin level were examined.

MATERIALS AND METHODS

1-Animals

Adult male Wistar rats, with a body weight (BW) of 200-250g, were obtained from central animal house of Hamadan University of Medical Sciences, Hamadan, Iran. Animals were housed in 12-hour light/dark condition in a constant temperature (21-23°C) and 55±10% relative humidity. Rats were fed with standard chow and water during the experiment (22,24). The protocol of this animal study was approved by the Ethics committee of Hamadan University of Medical Sciences.

2-Diabetes mellitus induction

To induce diabetes mellitus in rats, after overnight fasting, 120mg/kg Nicotinamide (NCD, N3376, Sigma) dissolved in normal physiological saline was injected intraperitoneally (IP). After 15 minutes, 60mg/kg Streptozotocin (STZ, S130, Sigma) dissolved in 0.1M citrate sodium buffer (pH=4.7), was administered IP. Diabetes mellitus induced with STZ-NCD is close to human type 2 diabetes mellitus; because nicotinamide exerts antioxidant effects and prevents the complete destruction of pancreatic beta cells. Seventy two hours after single IP injection of STZ-NCD, blood glucose (FBG) level was measured using a glucometer (Glucocard 01, Arkray, Japan). The animals with FBG level higher than 126mg/dl were considered as diabetic. The blood samples were taken from tail vein (1,22).

3-Experiment procedure

The animals were randomly divided into 8 groups of 5 animals each as given below. BCA (BCA, D2016, Sigma) was dissolved in 0.5% Dimethyl Sulfoxide (DMSO) for oral consumption and dissolved in 75% DMSO for IP injection. Oral administration was performed by using a gavage syringe (19).

Group 1: Healthy control (75% DMSO; IP), Group 2: Diabetic control (75% DMSO; IP)

Group 3: Diabetic + BCA (1mg/kg BW/day; IP), Group 4: Diabetic + BCA (5mg/kg BW/day; IP)

Group 5: Healthy control (0.5% DMSO; orally), Group 6: Diabetic control (0.5% DMSO; orally)

Group 7: Diabetic + BCA (10mg/kg BW/day; orally), Group 8: Diabetic + BCA (20mg/kg BW/day; orally)

Treatment was initiated 7 days after diabetes induction and continued for 30 days. FBG and BW were measured 3 times: before diabetes induction, the first and the last day of treatment. At the end of the treatment, animals were anesthetized by ether and then visceral adipose tissue samples were separated. The samples were immediately frozen

in liquid nitrogen and transferred into -80°C freezer (13). The blood samples were taken from the cardiac puncture; serum was separated and stored at -20°C for measuring insulin level.

4-Insulin assay

Insulin level was measured using rat insulin ELISA kit (ERINS, Thermo scientific). Insulin resistance index or homeostatic model assessment insulin resistance (HOMA-IR) was calculated as follows:

$$\text{Insulin}(\mu\text{U/ml}) \times \text{glucose}(\text{mg/dl}) / 405(16).$$

5-Total protein extraction

Tissue lysates were prepared on ice by using RIPA lysis buffer system (Sc-24948, Santa Cruz). A sample of frozen adipose tissue (0.2 g) was homogenized using 0.6 ml of RIPA buffer and 10 µl protease inhibitor. Homogenates were centrifuged at 4°C (14000 g, 10minutes×2). The insoluble fat layer was removed and the supernatants collected and stored at -20°C (7,10). Total protein concentration was measured using Bicinchoninic acid assay (Sc-202389, Santa Cruz).

6-Western blotting

The prepared samples containing equal amounts of total protein were run on 10%SDS-polyacrylamide gel electrophoresis (SDS-PAGE); and transferred to nitrocellulose membrane. The membrane was blocked with 5% skim milk in TBST (0.1% Tween 20 in Tris buffered saline, pH=7.6) for 1-hour at room temperature on a shaker. For immunoblotting, the membrane was incubated with primary antibodies against β-actin (1/1000, Sc-130657, Santa Cruz) or adiponectin(1/500, Sc-17044-R, Santa Cruz) in TBST at 4°C with agitation overnight. For removing the residual primary antibodies after immunoblotting, while agitation, the membrane was washed in TBST for 30 minutes. The immunoblots were incubated by 1/2000 dilution of horse-radish peroxidase (HRP) – conjugated secondary antibody (Sc – 2005, Santa Cruz) in TBST for 1-hour at room temperature with agitation. To remove the residual secondary antibodies, the membrane was washed using TBST, as mentioned above. At the end of the process, the protein bands were detected using an enhanced chemiluminescence (ECL) detection kit (Sc-2048, Santa Cruz). Densitometric analysis was performed by Image J Software(17,18,28).

7-Statistical analysis

The SPSS software was used for statistical analysis. The given values are expressed as mean±SD. In order to statistically compare the results between different groups, one way ANOVA followed by post hoc Tukeys' test was used. A p value less than 0.05 was considered significant in all statistical analysis.

RESULTS

The effect of IP injection of BCA on animals' body weight

Table 1 demonstrates the effect of IP injection of BCA on BW in STZ-NCD induced diabetic rats. BW increased significantly in the treated group by 5mg/kg BCA compared to diabetic control and 1mg/kg BCA treated rats (p<0.001), but it did not reach to BW of healthy rats (p<0.01). There was no considerable difference in BW between diabetic control and 1mg/kg BCA treated rats (p>0.05).

The effect of oral administration of BCA on animals' body weight

As is shown in Table 2, oral administration of BCA at both doses of 10 and 20 mg/kg, was able to increase the BW significantly compared to those of untreated diabetic rats (p<0.01). It should be noted that BCA at dose of 20mg/kg was more effective than 10mg/kg in weight gain (p<0.001). As can be inferred from Table 2, weight gain in healthy rats was considerably higher comparing to diabetic rats (p<0.001).

The effect of IP injection of BCA on FBG, insulin level and HOMA index

Table 3 indicates the effect of IP injection of BCA on FBG, insulin level and HOMA index in STZ-NCD induced diabetic rats. FBG decreased significantly in treated group with 5mg/kg BCA compared to diabetic control and 1mg/kg BCA treated rats (p<0.001). Injection of BCA at dose of 1mg/kg had no considerable effect on FBG level compared with diabetic untreated rats (p>0.05). Moreover, FBG level in healthy rats were not ably lower than that of treated diabetic rats (p<0.001). Diabetic rats (treated and untreated) showed no remarkable differences in insulin level (p>0.05). But HOMA index in animals treated by 5mg/kg BCA compared with diabetic control and 1mg/kg BCA treated rats, improved considerably (p<0.001). BCA at dose of 1mg/kg was not able to improve HOMA index

significantly ($p > 0.05$). At the end, diabetic rats and healthy rats were notably different in insulin level and HOMA index ($p < 0.001$).

The effect of oral administration of BCA on FBG, insulin level and HOMA index

Table 4 shows administration of BCA at both doses of 10 and 20 mg/kg was able to decrease the FBG level significantly compared to untreated diabetic rats ($p < 0.01$). Obviously, FBG level in animals which treated by dose of 20mg/kg was notably lower than animals which treated by dose of 10 mg/kg ($p < 0.001$), but it did not reach to FBG level of healthy rats ($p < 0.001$). Remarkable differences in FBG level were shown between healthy and diabetic rats ($p < 0.001$). Ten and twenty mg/kg oral administration of BCA could not affect insulin level in STZ-NCD induced diabetic rats ($p > 0.05$), but HOMA index improved notably at both doses compared with control diabetic rats ($p < 0.01$). It should be noted that dose of 20 mg/kg was significantly better than dose of 10 mg/kg in improving HOMA index ($p < 0.001$). As it is shown in Table 4, there were considerable differences between healthy and diabetic rats in HOMA index and insulin level ($p < 0.001$).

The effect of IP injection of BCA on adiponectin level in adipose tissue

Fig.1 demonstrates that adiponectin level was increased significantly ($p < 0.001$) only in animals which treated by dose of 5mg/kg BCA, in the other words, dose of 1mg/kg of BCA was not able to increase the adiponectin level ($p > 0.05$). The healthy, untreated diabetic rats, and diabetic rats that treated by dose of 1 mg/kg of BCA had approximately the same level of adiponectin ($p > 0.05$).

The effect of oral administration of BCA on adiponectin level in adipose tissue

According to Fig.2, adiponectin level was considerably higher in treated diabetic rats compared with untreated diabetic and healthy rats ($p < 0.01$). Dose of 20mg/kg was more effective in increasing the level of adiponectin than dose of 10mg/kg of BCA ($p < 0.001$). Healthy and untreated diabetic rats, had approximately the same level of adiponectin, in the other words, induction of diabetes did not affect the adiponectin level ($p > 0.05$).

DISSCUSION

Several studies reported that increased plasma level of adiponectin can improve insulin resistance in skeletal muscle cells(3,11,25,26). Adiponectin improves insulin resistance in skeletal muscle cells via the adaptor protein called APPL 1. Adipo Rsafter stimulation by adiponectin, facilitate the phosphorylation of APPL 1 in serine residue. Phosphorylated APPL1 joins with insulin receptor substrate (IRS) to make a complex which will facilitate the interaction between IRS and insulin receptor. Interaction between IRS and insulin receptor, will lead to phosphorylation of IRS in tyrosine residue. Phosphorylated IRS will phosphorylate and activate Akt protein in threonine residue. By activation of Akt and other proteins in signaling pathway of insulin, the number of Glut4 on the surface of skeletal muscle cells will increase and subsequently glucose uptake via Glut4 increases. In the other words, adiponectin facilitates interaction between insulin receptor and IRS; in this way insulin resistance, the main cause of type 2 diabetes mellitus, will improve. According to above, increasing plasma level of adiponectin, can be a therapeutic target in type 2 diabetic patients(3,4,8,14,15,21,23,25-27).

Results of the present study, consistent with the previous studies, showed that administration of BCA as a PPAR γ agonist can increase expression of adiponectin gene and adiponectin level significantly. Additionally, FBG level and HOMA index decreased at the end of the treatment. So it can be concluded that increasing the adiponectin level leads to insulin resistance improvement.

Masanori et al. reported the presence of a PPRE in the promoter of adiponectin gene; also PPAR γ agonists can increase the expression of adiponectin gene (26). Pita et al. indicated that Thiazolidinediones (TZDs), as PPAR γ agonists, improve insulin resistance via increasing adiponectin level in fat diabetic rats(21). Guo et al. reported that activation of PPAR γ via PPAR γ agonists, such as TZDs, can mediate anti-diabetic action via improving insulin resistance; however, the side effects of TZDs such as hepatotoxicity and heart failure limit the use of these compounds as anti-diabetic drugs(9). Wang et al. reported that BCA is a PPAR γ agonist and PPAR γ regulates glucose homeostasis(27). Harini et al. demonstrated that BCA administration in diabetic rats, can decrease FBG level(12). Azizi et al. indicated that BCA administration has hypoglycemic and anti-lipidemic effects in diabetic rats(2).

Table1: Effect of IP injection of BCA on the animals'body weight

	Healthy Control	Diabetic control	Diabetic + 1mg/kgBCA	Diabetic + 5mg/kg BCA
	(g)	(g)	(g)	(g)
Pre treatment day 0	242.8±16	248.5±9.1	243.6±6.6	245.2±14.1
7 days after diabetes induction	247.79±12.6	215.4±8.2	214.37±7	216.89±8.3
One month after treatment	298.5±9.9	167.4±12 ^{a*}	170.53±10 ^{a*}	260.32±14.4 ^{ab,b*,c*}

Mean±SDBW in healthy control (n=5), diabetic control (n=5) and BCA-treated rats (1 and 5mg/kg; n=5). a:compared with healthy control; b:compared with diabetic control; c:compared with diabetic rats treated by 1mg/kg BCA.#:p<0.01, *:p<0.001.

Table2: Effect of oral administration of BCA on the animals'body weight

	Healthy Control	Diabetic Control	Diabetic+ 10mg/kgBCA	Diabetic+ 20mg/kgBCA
	(g)	(g)	(g)	(g)
Pre treatment day 0	247.83±14.2	248.57±19.6	246.78±7.1	245.63±12.9
7 days after diabetes induction	252.51±15.5	221.57±11.7	218.16±14.1	215.53±6.6
One month after treatment	300.29±13.9	161.59±14.8 ^{a*}	200.71±19.2 ^{a*,b#}	268.54±14.1 ^{ab,b*,c*}

Mean±SD body weight in healthy control (n=5), diabetic control (n=5) and BCA-treated rats (10 and 20mg/kg; n=5). a:compared with healthy control; b:compared with diabetic control; c:compared with diabetic rats treated by 10mg/kg BCA.#:p<0.01, *:p<0.001.

Table3: Effect of IP injection of BCA on FBG and insulin level

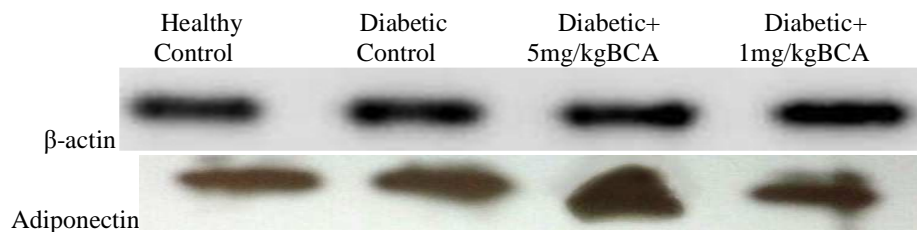
	Healthy control	Diabetic control	Diabetic+ 1mg/kg BCA	Diabetic+ 5mg/kg BCA
Pre treatment day 0FBG (mg/dl)	74.1±6.7	75.2±8.3	73.5±7.4	76.4±7.2
7 days after diabetes induction FBG (mg/dl)	71.3±7.2	244.2±16.3	247.04±12.5	248±13.1
One month after treatmentFBG (mg/dl)	72.2±6.8	245.6±14 ^{a*}	239±13.6 ^{a*}	185.8±14.2 ^{a*,b*,c*}
Insulin (µU/ml)	12.8±1.1	8.39±0.9 ^{a*}	8.58±0.8 ^{a*}	8.2±0.4 ^{a*}
HOMA-IR	2.36±0.3	5.17±0.9 ^{a*}	5.28±0.9 ^{a*}	3.45±0.6 ^{ab,b*,c*}

Mean±SD FBG, insulin and HOMA-IR in healthy control (n=5), diabetic control (n=5) and BCA-treated rats (1 and 5 mg/kg; n=5). a:compared with healthy control; b:compared with diabetic control; c:compared with diabetic rats treated by 1mg/kg BCA.#: p<0.01, *: p<0.001.

Table4: Effect of oral administration of BCA on FBG and insulin level

	Healthy control	Diabetic control	Diabetic+ 10mg/kg BCA	Diabetic + 20mg/kg BCA
Pre treatment day 0 FBG (mg/dl)	78.52±8.3	73.64±9.3	76.3±8.5	80.46±12.2
7 days after diabetes induction FBG mg/dl)	77±8.9	240.6±16.3	242.8±14	245.8±15.9
One month after treatment FBG (mg/dl)	73.8±7.3	252.73±9.4 ^{a*}	221.14±13.9 ^{a*,b#}	190.4±14.9 ^{a*,b*,c#}
Insulin(µU/ml)	13.8±0.81	8.36±0.73 ^{a*}	8.1±0.84 ^{a*}	8.25±0.91 ^{a*}
HOMA-IR	2.65±0.72	5.71±0.52 ^{a*}	4.22±0.78 ^{a*,b*}	3.87±0.64 ^{a*,b*,c#}

Mean±SD FBG, insulin and HOMA-IR in healthy control (n=5), diabetic control (n=5) and BCA-treated rats (10 and 20 mg/kg; n=5). a :compared with healthy control; b:compared with diabetic control; c:compared with diabetic rats treated by 10mg/kg BCA.#: p<0.01, *: p<0.001.



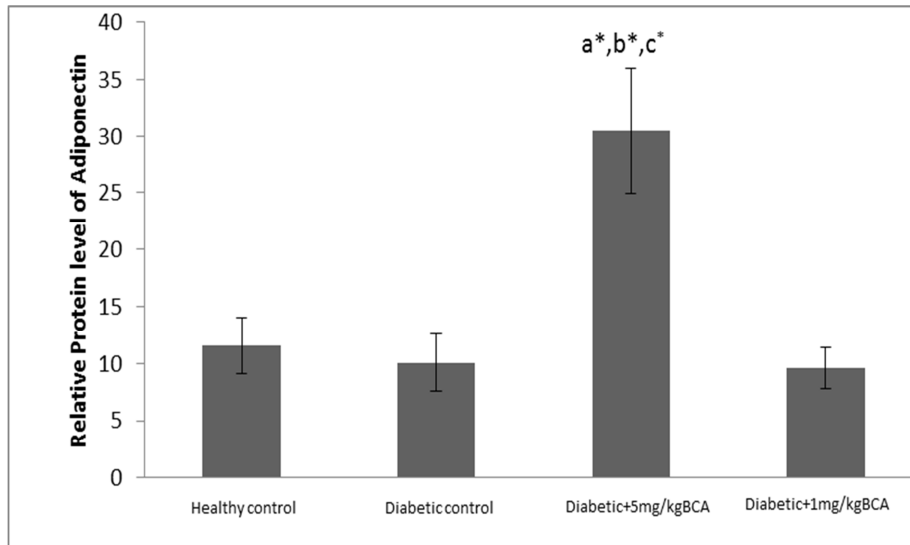


Fig.1. Results of western blot analysis of adiponectinin adipose tissue. Protein levels are expressed in arbitrary units after densitometric analysis. Bars represent the mean±SD. a: compared with healthy control; b: compared with diabetic control; c: compared with diabetic rats treated by 1mg/kg BCA.#: p<0.01, *: p<0.001

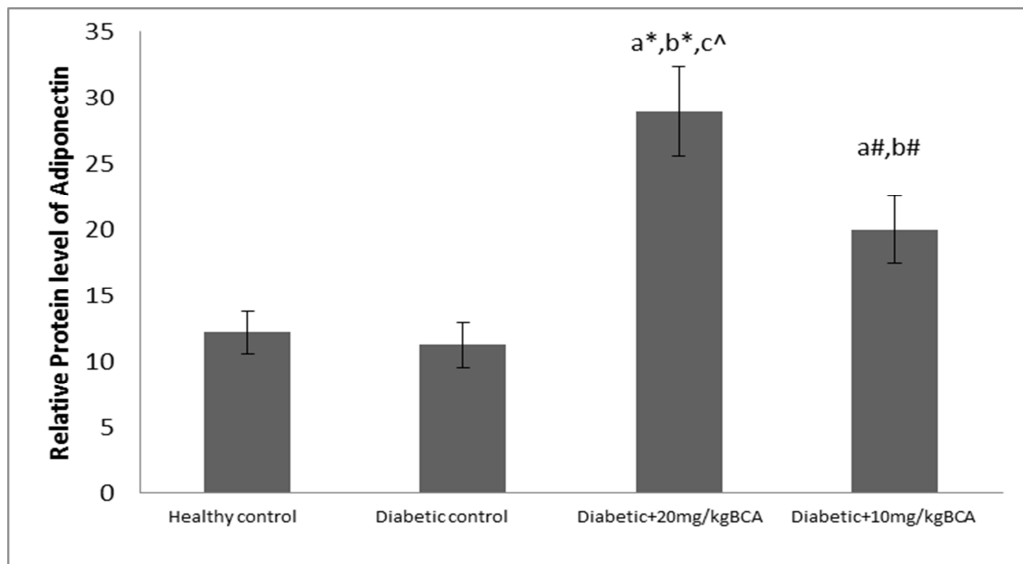
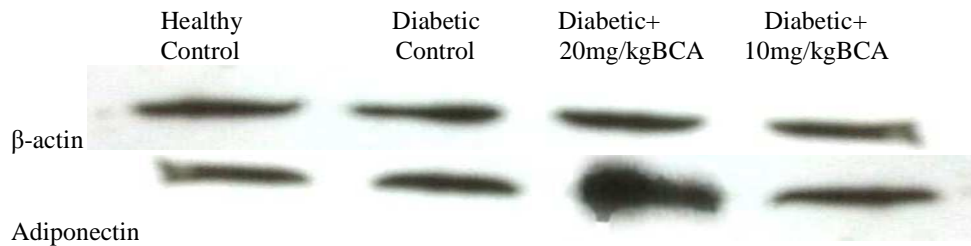


Fig.2. Results of western blot analysis of adiponectinin adipose tissue. Protein levels are expressed in arbitrary units after densitometric analysis. Bars represent the mean±SD. a: compared with healthy control; b: compared with diabetic control; c: compared with diabetic rats treated by 10mg/kg BCA. ^: p<0.05,#: p<0.01, *: p<0.001.

Based on the results of the present study, it can be deduced that BCA consumption in both orally and injection forms, is effective in lowering the blood glucose, so it can be used in both forms for controlling of type 2 diabetic

patients. More studies are needed to make Biochanin A an effective drug in type 2 diabetes mellitus treatment; most effective dose, the administration form and its side effects of prolonged use are still controversial. The present study was one of the first experiments that investigated the mechanism of BCA in lowering the blood glucose and improving the diabetes complications.

CONCLUSION

Results of our study indicated a dose dependent modulator effect of BCA on the expression of adiponectin adipose tissue of diabetic rats. Additionally, increased level of adiponectin was accompanied by decreased FBG and improved insulin resistance. Based on the findings of our study, BCA can be useful for control of type 2 diabetes mellitus; because it can improve insulin resistance; that is the main cause of type 2 diabetes mellitus.

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REFERENCES

- [1] Aboonabi, A., Rahmat, A., & Othman, F. 2014. Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. *Toxicology Reports*, 1: 915-922.
- [2] Azizi, R., Goodarzi, M. T., & Salemi, Z. 2014. Effect of biochanin A on serum visfatin level of streptozotocin-induced diabetic rats. *Iran Red.Crescent.Med.J.*, 16(9): e15424.
- [3] Caselli, C. 2014. Role of adiponectin system in insulin resistance. *Mol.Genet.Metab*, 113(3): 155-160.
- [4] Cheng, K. K. Y., Lam, K. S. L., Wang, B., & Xu, A. 2014. Signaling mechanisms underlying the insulin-sensitizing effects of adiponectin. *Best Practice & Research Clinical Endocrinology & Metabolism*, 28(1): 3-13.
- [5] Esfahani, M., Movahedian, A., Baranchi, M., & Goodarzi, M. T. 2015. Adiponectin: an adipokine with protective features against metabolic syndrome. *Iran J Basic Med Sci*, 18(5): 430-442.
- [6] Ezuruike, U. F. & Prieto, J. M. 2014. The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. *J.Ethnopharmacol.*, 155(2): 857-924.
- [7] Fouad, M. M., Pelletier, M., Boulet, M. M., Mayrand, D., Brochu, G., Lebel, S., Poirier, D., Fradette, J., Cianflone, K., Luu-The, V., & Tchernof, A. 2015. Oxidative activity of 17beta-hydroxysteroid dehydrogenase on testosterone in male abdominal adipose tissues and cellular localization of 17beta-HSD type 2. *Mol.CellEndocrinol*, 414: 168-176.
- [8] Goodarzi, M. T., Babaahmadi-Rezaei, H., Kadkhodaei-Eliaderani, M., & Haddadinezhad, S. 2007. Relationship of serum adiponectin with blood lipids, HbA(1)c, and hs-CRP in type II diabetic postmenopausal women. *J Clin.Lab Anal.*, 21(3): 197-200.
- [9] Guo, L. & Tabrizchi, R. 2006. Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance. *Pharmacol.Ther.*, 111(1): 145-173.
- [10] Gupte, A. & Mora, S. 2006. Activation of the Cbl insulin signaling pathway in cardiac muscle; dysregulation in obesity and diabetes. *Biochem.Biophys.Res.Commun.*, 342(3): 751-757.
- [11] Hammarstedt, A., Andersson, C. X., Rotter, S., V., & Smith, U. 2005. The effect of PPARgamma ligands on the adipose tissue in insulin resistance. *Prostaglandins Leukot.Essent.Fatty Acids*, 73(1): 65-75.
- [12] Harini, R., Ezhumalai, M., & Pugalendi, K. V. 2012. Antihyperglycemic effect of biochanin A, a soy isoflavone, on streptozotocin-diabetic rats. *Eur.J.Pharmacol.*, 676(1-3): 89-94.
- [13] Ibrahim, M. M. 2010. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes.Rev.*, 11(1): 11-18.
- [14] Iwaki, M., Matsuda, M., Maeda, N., Funahashi, T., Matsuzawa, Y., Makishima, M., & Shimomura, I. 2003. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes*, 52(7): 1655-1663.
- [15] Izadi, M., TaghiGoodarzi, M., SamariKhalaj, H., Khorshidi, D., & Doali, H. 2011. Serum Adiponectin Levels are Inversely Correlated with Insulin Resistance in Obese Men with Type 2 Diabetes. *Int J EndocrinolMetab*, 9(1): 253-257.
- [16] Katsuki, A., Sumida, Y., Gabazza, E. C., Murashima, S., Furuta, M., Araki-Sasaki, R., Hori, Y., Yano, Y., & Adachi, Y. 2001. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. *Diabetes Care*, 24(2): 362-365.
- [17] Li, Y., Qin, G., Liu, J., Mao, L., Zhang, Z., & Shang, J. 2014. Adipose tissue regulates hepatic cholesterol metabolism via adiponectin. *Life Sci*, 118(1): 27-33.

- [18] Maillard, V., Uzbekova, S., Guignot, F., Perreau, C., Rame, C., Coyral-Castel, S., & Dupont, J. 2010. Effect of adiponectin on bovine granulosa cell steroidogenesis, oocyte maturation and embryo development. *Reprod.Biol.Endocrinol*, 8: 23.
- [19] Moon, Y. J., Sagawa, K., Frederick, K., Zhang, S., & Morris, M. E. 2006. Pharmacokinetics and bioavailability of the isoflavonebiochanin A in rats. *AAPS.J*, 8(3): E433-E442.
- [20] Peplow, P. V. 2015. Repeated electroacupuncture in obese Zucker diabetic fatty rats: adiponectin and leptin in serum and adipose tissue. *J Acupunct.Meridian Stud*, 8(2): 66-70.
- [21] Pita, J., Panadero, A., Soriano-Guillen, L., Rodriguez, E., & Rovira, A. 2012. The insulin sensitizing effects of PPAR-gamma agonist are associated to changes in adiponectin index and adiponectin receptors in Zucker fatty rats. *Regulatory Peptides*, 174(1-3): 18-25.
- [22] Rezaei, F. A., Saidijam, M., Goodarzi, M. T., YadegarAzari, R., Asadi, S., Zarei, S., & Shabab, N. 2015. Effect of Resveratrol Supplementation on the SNARE Proteins Expression in Adipose Tissue of Streptozotocin-Nicotinamide Induced Type 2 Diabetic Rats. *Iran J Med Sci*, 40(3): 248-255.
- [23] Sharabi, Y., Oron-Herman, M., Kamari, Y., Avni, I., Peleg, E., Shabtay, Z., Grossman, E., & Shamiss, A. 2007. Effect of PPAR- γ Agonist on Adiponectin Levels in the Metabolic Syndrome: Lessons From the High Fructose Fed Rat Model. *American Journal of Hypertension*, 20(2): 206-210.
- [24] Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res.Clin.Pract.*, 87(1): 4-14.
- [25] Suzuki, S. T., Zhao, B., & Yang, J. 2008. Enhanced muscle myostatinpropeptide increases adipose tissue adiponectin, PPAR-alpha, and PPAR-gamma expressions. *Biochem.Biophys.Res.Commun*, 369(2): 767-773.
- [26] Tsao, T. S., Lodish, H. F., & Fruebis, J. 2002. ACRP30, a new hormone controlling fat and glucose metabolism. *European Journal of Pharmacology*, 440(2-3): 213-221.
- [27] Wang, L., Waltenberger, B., Pferschy-Wenzig, E. M., Blunder, M., Liu, X., Malainer, C., Blazevic, T., Schwaiger, S., Rollinger, J. M., Heiss, E. H., Schuster, D., Kopp, B., Bauer, R., Stuppner, H., Dirsch, V. M., & Atanasov, A. G. 2014. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARgamma): a review. *Biochemical Pharmacology*, 92(1): 73-89.
- [28] Ziamajidi, N., Khaghani, S., Hassanzadeh, G., Vardasbi, S., Ahmadian, S., Nowrouzi, A., Ghaffari, S. M., & Abdirad, A. 2013. Amelioration by chicory seed extract of diabetes- and oleic acid-induced non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) via modulation of PPARalpha and SREBP-1. *Food Chem.Toxicol.*, 58: 198-209.
- [29] Zimmet, P., Alberti, K. G. M. M., & Shaw, J. 2001. Global and societal implications of the diabetes epidemic. *Nature*, 414(6865): 782-787.