

Antidiabetic and Synergistic Effects of Anthocyanin Fraction from *Berberis integerrima* Fruit on Streptozotocin-Induced Diabetic Rats Model

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Abstract

Diabetes mellitus is a complex endocrine disorder. There is a serious attempt to identify antidiabetic compounds from natural sources to use with other drugs for reduction of diabetes complications. Present study is based on the investigation of antihyperglycemic effect of anthocyanin fraction of *Berberis integerrima* Bunge (AFBI) fruits on some physiological parameters (glucose level, glycogen content, and body weight) in normal and streptozotocin-induced (STZ-induced) diabetic rats and evaluation of synergic effect of this fraction with metformin and glibenclamide. Male Sprague dawley rats were divided into nine groups: healthy control group, diabetic control group, diabetic groups treated with anthocyanin fraction (200, 400 and 1000 mg/kg, respectively); diabetic groups treated with glibenclamide and metformin separately, diabetic groups treated with glibenclamide + anthocyanin fraction (1000 mg/kg), metformin + anthocyanin fraction (1000 mg/kg). Treatment of diabetic rats with AFBI (400, 1000mg/kg) significantly decreased blood glucose as compared with control. Moreover, AFBI (400, 1000mg/kg) significantly increased liver glycogen and body weight compared to control. Nevertheless, there were no synergistic effects between anthocyanin fraction and metformin or glibenclamide on blood glucose, liver glycogen, and body weight. The results of this study indicate that AFBI possesses hypoglycemic effects and may be considered for evaluation in future diabetes clinical studies.

Keywords: Antidiabetic, *Berberis Integerrima* Bunge, Synergistic Effects, Streptozotocin.

1. Introduction

Diabetes mellitus refers to a group of metabolic disorders, in which inadequate insulin secretion and/or insulin action leads to hyperglycemia (1). Chronic hyperglycemia can lead to a variety of tissue damages. The kidneys, eyes, nerves, blood vessels, and heart are the organs most exposed to the risk of chronic hyperglycemia damages (2).

It has been proposed that hyperglycemia may cause free radicals formation, oxidative stress, and deficiency of endogenous antioxidant defense system, which are in charge of

diabetes complications (1, 3). Recently, there is a great interest in the role of natural antioxidants to diminish damage of oxidative stress and diabetes associated complications (4).

Anthocyanin belong to flavonoids group, which are not only natural pigments, but also have strong antioxidant capacities. They are able to be effective antioxidants by enzymatic and non-enzymatic mechanisms. These properties make them as proper candidates in treatment of many diseases, such as cardiovascular diseases (5). Among different plants, *Berberis* sp. are rich in anthocyanin and are known as anthocyanin sources (6). *Berberis integerrima* Bunge belongs to Berberidaceae, which is famous as an antidiabetic plant in Persian folk

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medicine (based on ethnopharmacology study).

The aim of this study was evaluation of the effect of this fraction on glucose level, glycogen content, body weight, and synergic effect study of anthocyanin fraction with glibenclamide (G3), as well as metformine (M15) in STZ-induced diabetic male rats.

2. Materials and method

2.1. Chemicals

All the chemicals used were obtained from Sigma and Aldrich Company (St. Louis, MO, USA).

2.2. Plant material

Fruits of *Berberis integerrima* Bunge was manually harvested on September 2012, from Kohmar (120 km from Shiraz, Capital of Fars Province, Iran), and were identified and authenticated (voucher no.Pm396) in the museum of medicinal plants, department of pharmacognosy, Shiraz University of Medical Sciences, Shiraz, Iran.

2.2.1. Extraction

The fruit (700 g) was freeze-dried, then dried by freeze dryer and ground into powder. The powder was percolated with ethanol for 48 h, and the extract was concentrated in rotary evaporator under vacuum at 40 °C followed by speed vacuum.

A portion of the crude extract was then suspended in 0.3% TFA (0.3 ml in 100 ml water), filtered, and shaken well with ethyl acetate in decanter. The aqueous phase was collected. This extraction process was repeated three times. The resultant phase was then loaded in an amberlite column, and rinsed with distilled water containing 0.3% TFA to delete polysaccharides. The anthocyanin phase was eluted with 0.3% TFA in methanol, then concentrated by rotary evaporator. The yields of extraction and anthocyanin fractionation were 38.4% and 11.85%, respectively.

2.3. Animals

Adult male sprague dawley rats (200-300 g) were prepared from the animal house of Shiraz University of Medical Sciences Shiraz, Iran. All animals were maintained in 12/12 h light/dark cycle at 21±2 °C. They were kept for two weeks pri-

or to experimentation, to adapt with experimental condition, while food and water were accessible.

2.3.1. Study design and doses

45 rats were selected and divided into 9 groups of five each. Diabetes was induced in 16-hour fasted rat by single injection of fresh STZ solution (60 mg/Kg, i.p.) in 8 groups, while control group was injected with normal saline.

Blood samples were measured by tail vein sampling after 10 days to confirm rendered diabetes in rat. Animals with fasting blood glucose levels above 300 mg/dl were considered to have diabetes.

The rats were divided into nine groups of comprising 5 randomly animals in each group as follows: group 1, healthy control group treated with normal saline and 10% ethanol; group 2, diabetic control group treated with normal saline and 10% ethanol; Group 3, diabetic group treated with anthocyanin fraction (200 mg/kg); Group 4, diabetic group treated with anthocyanin fraction (400 mg/kg); group 5, diabetic group treated with anthocyanin fraction (1000 mg/kg); group 6, diabetic group treated with glibenclamide (3 mg/kg); group 7, diabetic group treated with metformin (15 mg/kg); group 8, diabetic group treated with glibenclamide (3 mg/kg) and anthocyanin fraction (1000 mg/kg); group 9, treated with metformin (15 mg/kg) and anthocyanin fraction (1000 mg/kg). After the end of 14 days, they were fasted for 16 hours; then blood glucose levels were measured. Then the rats were scarified under thiopental anesthesia, livers were collected and weighed and liver glycogen was calculated.

2.4. Anthocyanin content measurement

The spectrophotometric pH differential method was used to quantify anthocyanins in the collected fractions (7). Briefly, 0.4 ml of sample was mixed with 3.6 ml of corresponding buffers. Buffers were made with 0.025 M potassium chloride solution and 0.4 M sodium acetate solution adjusted to pH 1.0 and 4.5 with HCl, respectively. The absorbance of each solution was measured at 519 nm against a distilled water blank, using an UV-visible spectrometer (PG Instruments Ltd, T-80+). The anthocyanin content

was calculated by the following equation: Eq 1.

$$\text{Anthocyanin Content (mg/L)} = (A \times MW \times DF \times 1000) / \epsilon \times L \quad \text{Eq1.}$$

where A is (A519 (pH1.0) - A519 (pH4.5)), MW is molecular weight of anthocyanin (433.2 g/mol), DF is dilution factor (10), ϵ is extinction coefficient ($31600 \text{ L cm}^{-1} \text{ mol}^{-1}$) and L is the path length (1 cm).

2.5. Extraction of glycogen from liver

Glycogen extraction and measurement was performed by Trichloroacetic Acid (TCA) according to butlet et.al method with slight modifications (8). Rat livers were isolated; the proper volume of TCA (10%) was added and homogenized for 30 minutes. Then, the homogenate was centrifuged for 5 min. The supernatant was collected and ethanol (95%) was added to observe the glycogen particles. The solution temperature was increased up to 50 °C and NaCl was added to obtain more glycogen particles. After 5 min, it was centrifuged and the precipitate was dissolved in distilled water and ethanol was added, then centrifuged again. Ethanol was added to precipitate; then ethanol was evaporated and the remained powder was collected and their weight was measured. Finally glycogen content was reported as mg per weight of liver (g).

2.6. Statistical analysis

All the reported data are presented as mean \pm SD of three experiments. Statistical analysis was performed using one-way ANOVA and T-test followed by LSD post-tests by SPSS (version 20). Differences were considered statistically significant at a $P < 0.05$.

3. Results

3.1. Anthocyanin analysis

The anthocyanin content in anthocyanin fraction was 10.2 ± 0.65 mg per g of fraction, which is reported as the average of three tests.

3.2. Antihyperglycemic activity

Streptozotocin is a chemical toxin able to induce the immune system against Langerhans islets beta cells; and 60 mg/kg STZ lead to emergence of clinical diabetes within 2-4 days (9). In this study, diabetes was induced by single injection (IP) of STZ (60 mg/kg) in rats.

According to Fig.1, glucose measurements in all groups showed that after diabetes induction by a single dose of STZ, blood glucose significantly increased in comparison to the healthy control. Results indicate that diabetes was successfully induced by STZ. Treatment of diabetic rats with the anthocyanin fractions (200, 400 and 1000 re-

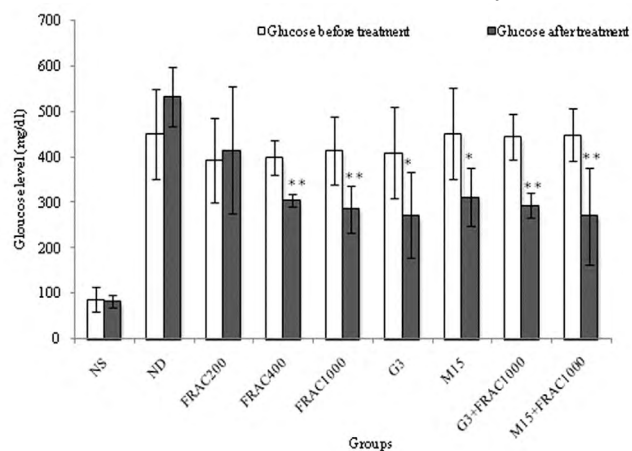


Figure 1. Effect of anthocyanin fraction on the glucose level (mg/dL) in normal (healthy control) and diabetic rats before and after treatment. (* $P < 0.05$ glucose level compared to after treatment.

** $P < 0.01$, glucose level compared to before treatment. Abbreviations: NS: healthy control group treated with normal saline and 10% ethanol; ND: diabetic control group, FRAC200: diabetic group treated with anthocyanin fraction (200 mg/kg); FRAC400: diabetic group treated with anthocyanin fraction (400 mg/kg); FRAC1000: diabetic group treated with anthocyanin fraction (1000 mg/kg); G3: diabetic group treated with glybenclamide (3 mg/kg); M15: diabetic group treated with metformin (15 mg/kg); G3+FRAC1000: diabetic group treated with glybenclamide (3 mg/kg) and anthocyanin fraction (1000 mg/kg); M15+FRAC1000 group 9, treated with metformin (15 mg/kg) and anthocyanin fraction (1000 mg/kg).

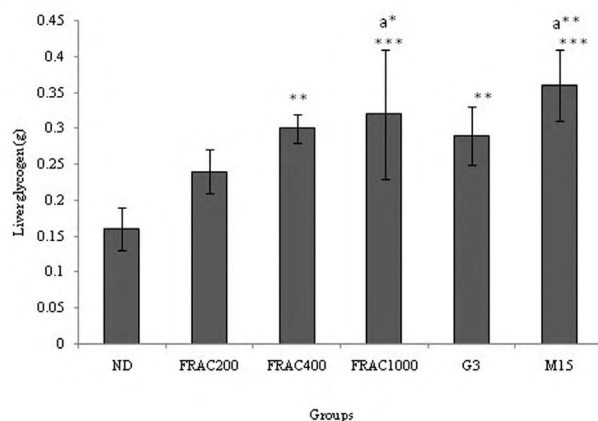


Figure 2. Hepatic glycogen levels (mean±SD) in different groups treated by drugs and different concentration of anthocyanin fraction ***: $P<0.001$ and **: $P<0.01$ compared to diabetic control, a**: $P<0.01$ and a*: $P<0.05$ compared to group receiving anthocyanin fraction (200 mg/ml).

duced glucose level 28.3%, 42.7%, and 46.4%, respectively as compared to untreated diabetic rat. It seems that the antihyperglycemic effects of these fractions were increased with a concentration increase from 200 to 400 mg/kg; but there is no significant difference between 400 mg/kg and 1000 mg/kg effect.

Results of metformin and glibenclamide treated rats indicated that blood glucose level decreased 41.3% and 48.9% as expected. In the diabetic groups receiving glibenclamide+anthocyanin fractions (1000 mg/ml) glucose reduced 44.9%; and diabetic rats treated by metformine+anthocyanin fractions (1000 mg/ml) glucose level diminished 49.2% in comparison to untreated diabetic rats.

3.3. Liver glycogen content

Liver glycogen content in the diabetic rat

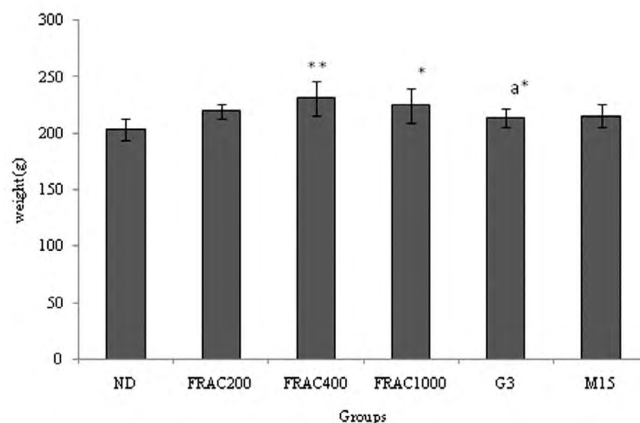


Figure 3. Body weight (mean±SD) in different groups treated by drugs and different concentrations of anthocyanin fraction. **: $P<0.001$ and *: $P<0.05$ compared to diabetic control, a*: $P<0.05$ compared to the groups receiving anthocyanin fraction (200 mg/ml).

group treated with AFBI (1000 mg/kg) was significantly higher than that of the rat treated with AFBI (200mg/ml) ($P<0.05$). Hepatic glycogen of the diabetic rat groups receiving AFBI (1000 mg/kg) and metformine was significantly higher than diabetic control ($P<0.001$). Moreover, AFBI (400 mg/kg) and glibenclamide treatment increased liver glycogen of the diabetic rat groups in comparison to diabetic control ($P<0.01$) (figure 2).

3.4. Effect on body weight of rats

In the diabetic control body weight was reduced 10.4% in comparison to healthy control, because of STZ injection. Anthocyanin fraction (400 mg/kg) is the most effective agent on body weight increase in comparison to diabetic control ($P<0.01$). Weight of this group was even more than normal group. Weight of the animals in the dia-

betic group receiving anthocyanin fraction (1000 mg/kg) was significantly ($P < 0.05$) more than that of the control group.

Nevertheless, there was no considerable difference between the weight of the diabetic groups receiving glibenclamide and metformin and those of the diabetic control group.

3.5. Synergic effects

It seems that although AFBI improves glucose level, liver glycogen, and body weight in diabetic induced rats, there is no synergic effect among this fraction and glibenclamide, as well as metformin in diabetes.

4. Discussion

Diabetes mellitus is a metabolic disorder in which uncontrolled hyperglycemia is the basis of several complications affecting longevity and life quality (3, 10). In traditional medicine, barberry's fruits are applied as an antidiabetic drug (11), which contain high amount of barberry anthocyanin (5). Anthocyanin pigments are globally well known as medicinal agents. Radical scavenging is the most famous properties of anthocyanins (5). Result of our study showed anthocyanin content of these fruits are comparable to anthocyanin content of *Nasturtium (Tropaeolum majus)* flowers (12), fruits of blackberries (*Rubus glaucus*) (13) and a hybrid of strawberries (*Fragaria ananassa*) (14).

In the current study, we investigated the effect of antocyanin fraction on glucose level of diabetic rats. For this purpose, STZ (60 mg/kg) was used as diabetes inducer in rat and caused a significant increase inserum glucose, which is consistent with previous findings (15). Streptozotocin is able to increase xanthine oxidase substrate, and subsequently induce formation of superoxide, hydrogen peroxide, and hydroxyl radicals, which finally leads to experimental diabetes in animals (15).

Results of the present study showed metformin (15 mg/kg) and glibenclamide (3 mg/kg) were able to modifyserum glucose in treated rats.

The anthocyanin fraction (400 and 1000 mg/kg) treated group, glucose levels were reduced as compared to the diabetic control group. In Groups receiving both anthocyanin fraction (1000 mg/kg)+(metformin or glibenclamide), glucose was

decreased significantly. The maximum reduction in glucose levels was seen in the group receiving both fraction (1000 mg/kg) and metformin (49.2%). These results were consistent with the result of Grace et.al, in which anthocyanin fraction of low-bush blueberry reduced glucose significantly (16).

A number of recent reports exhibited potential benefits of anthocyanin for diabetes by different mechanisms. For instance, antioxidant properties of anthocyanins can protect pancreatic β -cells against oxidative stress (17, 18). Insulin release from pancreatic β -cells by anthocyanins is another probable cause of their antidiabetic effect (18, 19). Furthermore, anthocyanin help to raise the phosphorylation of AMP activated protein kinase (AMPK) and induce enzyme activation (18). AMPK is a kind of sensing enzymes, which is activated by cellular stresses. AMPK activation is a major mechanism for enhancing glucose transport into skeletal muscles. Subsequently, AMPK activation leads to transport and translocation of glucose transporters in muscles and decreased blood glucose levels (18). The ability of anthocyanin to inhibit alpha glucosidase in small intestine is considered as one of their antidiabetic effects (20). So, in brief, antioxidant activity (17, 18), release of insulin (18, 19), inhibition of starch and disaccharides break-down in intestines (20), and induction of glucose transport to muscles (18) are the recognized mechanisms of anthocyanins for glucose reduction. Glycogen is the storage form of glucose. Liver and skeletal muscles are two main tissues for glycogen storage. Insulin not only stimulates glycogen synthase, but also inhibit glycogen phosphorylase (21). In diabetic rats, the change in glucose synthesis leads to a reduction of glycogen content (22). In this study, treatment with anthocyanin fraction (400 and 1000 mg/ml) significantly increased the total glycogen content in the liver tissue compared to the control group in diabetic rats. These effects were approximately similar to the results observed with glibenclamide and metforin treatments; however, adding anthocyanin fraction (1000 mg/ml) to these drugs (glibenclamide and metformin) did not show any significant effect on the glycogen content. So, the ability of anthocyanin fraction to improve glycogen levels indicates that this fraction has the potential to be an antidia-

betic agent.

Obesity is considered as a complex condition, strongly related to insulin resistance and lipid metabolism in peripheral tissues (23). Therefore, in this study body weight was considered as a measurable factor. Results of body weight measurements exhibited significant increase in diabetic control weight in comparison to healthy control because of STZ injection. In groups receiving 400 and 1000 mg/ml anthocyanin fractions, body weight increased significantly compared to diabetic control. Our results support the previous results that showed anthocyanin from purple corn prevented obesity in mice fed with a high-fat diet in comparison to a group received same diet without anthocyanins. Moreover, the effect of anthocyanin on obesity is related to the enhancement of circulating free fatty acids and tumor necrosis factor (TNF- α). It seems that antioxidant administration increases proinflammatory cytokines (i.e., TNF- α), which leads to inhibit insulin signaling and pancreatic beta cell function (23). Another possible antiobesity function of anthocyanins may be related to down-regulations of the enzymes participated in the fatty acid and triacylglycerol synthesis (23) and up-regulation of adiponectin in

white adipose tissue, which regulates glucose level as well as fatty acid breakdown (24,25).

Briefly, the gene expression profile analysis of the human adipocytes treated with anthocyanin exhibited significant changes in adipocytokine expression and a strong induction of lipid metabolism related genes in the treatment groups (25).

In conclusion, anthocyanin fraction of *Berberis integerrima* Bunge fruits indicated powerful antihypoglycemic effect in diabetic model rats. Glucose level reduction is not the only effect of these anthocyanin; they also improve liver glycogen and body weight, especially at doses of 400 and 1000 mg/Kg. The high anthocyanin content of *Berberis integerrima* Bunge fruits suggests them as a considerable antidiabetic agent. Further investigations must be conducted to investigate other biological effects of these compounds.

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Conflict of Interest

None declared.

5. References

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