Effects of Hydro-Alcoholic Leaf Extract of Kardeh (*Biarum Bovei Blume*) on the Blood Glucose and Lipid Peroxidation in Cerebral Tissues and Lipid Profile in Streptozotocin Induced Diabetic Rats

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Abstract

Objective: Oxidative stress is the result of an imbalance between free radical production and antioxidant defenses of the body. The use of antioxidants can have preventive effects in diabetes. The present study aims to show the effects of Biarum Bovei extracts on lipid profile, lipid peroxidation in streptozocin induced diabetic male rats.

Materials and Methods: Animals were randomly assigned to the control group, diabetic with streptozotocin (STZ 60 mg/kg) and 5 diabetic groups rats which have received hydro-alcoholic extract *Biarum Bovei* (50, 100, 200, 400, 800 mg/kg) via gavages for two weeks. Blood glucose, serum lipid profile and malondialdehyde (MDA) in brain tissue were measured at the end of experiment.

Results: The results indicate that blood glucose in diabetic group increased significantly ($P<0.0001$) in comparison with the control group. Biarum Bovei extract at a dose (100 and 200 mg/kg), causing a significant decrease in blood glucose levels ($P<0.01$). The MDA significantly decreased in the hippocampus in the group receiving 100, 200 mg/kg Biarum Bovei extract ($P<0.001$, $P<0.01$) respectively.

Conclusion: Hydro-alcoholic extract of *Biarum Bovei* may have antioxidant properties which reduce blood glucose and complications of diabetes such as lipid peroxidation and lipid profiles. It can be used from this plant to improve diabetes along with medication.

Keywords: *Biarum Bovei*, Blood glucose, Lipid peroxidation.

Introduction

Variations in the glucose and insulin levels following streptozotocin (STZ) transfusion is a result of the abnormal pancreas beta cells functioning. STZ causes a disorder in the glucose oxidation, and reduces blood insulin production and secretion (1). In this molecular path, the free radical resulting from the hydrogen super-oxidation, per-oxidation, the hydroxyl radical and the other active oxygen types generated as a consequence of the STZ toxicity (1). The lipids existing in the cell membranes are one of the free radicals targets. The free radicals damage the cells through membrane phospholipids per-oxidation, dissolving the two-layer cell membrane and affecting the membrane proteins function (2). Although the cell lipids are regenerated very rapidly under

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natural conditions but many of the products resulting from the lipids peroxidation such as hydroperoxidase, alcohol and aldehydes are effective on the cells' biological structure and behavior (3). Malondialdehyde (MDA) is an organic compound (CH_2 $(CHO)_2$). It is an active aldehyde and active electrophile species. MDA is the final lipid oxidation species. MDA produces a red fluorescent derivative with thiobarbitoric acid (TBAR) which can be assayed by spectrophotometer. Oxidative stress not only causes an increase in the production of the free radicals it also causes a reduction in the cellular anti-oxidative mechanism (4). Hyperglycemia causes non-enzymatic glycosylation of enzymes and proteins that eliminate free radicals. (5). According to the lipid hypothesis, abnormal cholesterol levels (hypercholesterolemia) actually higher concentrations of Low Density Lipoprotein (LDL) and lower concentrations of functional High Density Lipoprotein (HDL) are strongly associated with cardiovascular diseases because of promoting Atheroma development in arteries (atherosclerosis) (6)In diabetic patients the hyperlipidemia is prevalent (7). Typical findings are total and very low density lipoprotein (VLDL) cholesterol, triglyceride concentration increasing, decreasing of HDL and predominance of small, dense LDL particles (8).

Alot of attention has been paid to use of natural antioxidants. (6). *Biarum Bovei* is a plant from the order Araceae. One species of the like is *Biarum Bovei* which is an edible, delicate plant with broad leaves. Its habitat is scattered in Turkey, Syria, Iran and Iraq (9,10). The presence of the Flavonoids and Anthocyanins in Aracea was first reported by Williams et al (1981) (11). Also, this order has been justified to have existence of alkaloids and Amins, saponins, cinnamic acids and flavonoids (12). Flavonoids are consisted of a wide spectrum of the polyphenolic compounds which are distributed extensively in photosynthesizing cells and they are known for their numerous pharmacological activities (13). There is a linear relationship between plant extracts antioxidant activities and their phenolic contents and it has been well recognized that the phenolic compounds have antioxidant activities that can protect the cells against oxidative reactions (14). As it is indicated in the experiments that the antioxidants are effective in both preventing diabetes and in curing the diabetes symptoms, we studied the *Biarum Bovei* extract effects on the blood glucose levels, lipid peroxidations and lipid profile in diabetic model male rats.

Materials and Methods

Forty-nine adult male Wistar rats (220±30 g) were obtained from Central Animal House of Jundishapur University of Medical Sciences, Ahvaz, Iran. They were housed individually in standard cages and maintained in a temperature-controlled room (21±2°C) on a 12/12-h light/dark cycle, humidity of (50%- 55%) with free access to the food and water and they were randomly divided into 7 groups (n=7). These groups were divided as control and diabetic groups received 60 mg/kg-STZ and five remained groups resaved 60 mg/kg-STZ-induced diabetic groups which were fed on *Biarum Bovei* extracts (50, 100, 200, 400, 800 mg/kg) by the use of Gavages method for two weeks. The study methodology was confirmed by the university committee based on the international regulations regarding the laboratory animals.

Diabetic animals

Diabetes was induced by a single dose of streptozotocin (sigma aldrich) STZ (60 mg/kg) intraperitoneally. 72 hr after STZ administration, blood was taken from lateral veins of the tail. The rise in blood glucose by glucometer (Bionime rightest GM110 KMT, Switzerland) was confirmed. Blood glucose level above 200 mg/dl were considered as diabetes(15)

Preparation *Biarum Bovei* Extract

Biarum Bovei plant was collected in early spring from the surrounding of Izeh city, Iran,

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and then its leaves were separated and dried in open air under shade for 2 weeks. After drying, the leaves were powdered (with diameter of less than 0.4 mm) with an electric grinder and *Biarum Bovei* powder was rinsed in ethanol 72% for 3 days at room temperature. *Biarum Bovei* powder and ethanol mix were stirred. Then alcohol and powder mix were finely filtered to obtain its extract. The obtained extract was distilled in vacuum to evaporate its alcohol completely. Finally, *Biarum Bovei* Extract was obtained (yield 28%) as a brown powder after full evaporation (16).

Tissue MDA measurement

Each rat brain was separated as cortex, cerebellum, striatum and hippocampus. Those were weighted and for every 1g of the tissue there was poured 10 ml 1.5% KCL solution in the big test tube. About 0.5 ml of the homogenized solution was picked up and transferred to the test tube. 2.5 ml 3% TCA (Trichloroacetic acid) was added to each of the 6 test tubes and it was kept for 10 minutes in 37° C water bath. The tubes were centrifuged for ten minutes in 3000 r/m. 0.5 ml of the surfactant solution was taken. It was transferred to 6 secondary test tubes with 3 ml 1% phosphoric acid and 1 ml 0.67% TBA (Thiobarbitoric acid) solution was added. It was placed in boiling water for 45 minutes. The tubes were chilled down in an ice container and they were added wit 4 ml butanol. After steering the solution, the tubes were instantly centrifuged in 3500 r/m and in the end the absorption waves were read in a 532 nm wavelength (17).

Standard curve

At first there is a need for the MDA standard solution to be prepared and then the wavelength can be measured by making use of the spectrophotometer device and in the end the standard curve can be mapped for it. 0.5 ml standard solution with 0.5, 1, 2, 4, 6, 8 and 10 micromole concentrations was picked up and transferred to 6 test tubes. Then 3 ml 1% phosphoric acid solution was added to each of the test tubes and the remaining stages were repeated as specified previously. The absorption was read in a 532 nm wavelength (17).

The current study data have been presented in a Mean ± SEM format and then they were analyzed through the use of appropriate statistical tests in Excel and SPSS environments and by taking advantage of one-way ANOVA method and Tukey's range test and the discrepancies obtained in the results derived for various groups was considered to be statistically significant in $P<0.05$ level.

Studying lipid profile

Serum levels of glucose, triglyceride (TG), cholesterol (C) and HDL were determined by kits purchased from Biological-Chemical Company. Iran. LDL and VLDL were also calculated: *LDL= Cholesterol (C) – (HDL+VLDL) and VLDL=TG/5(18).*

Results

Biarum Bovei effect on the blood sugar (BS) level

The results of current study indicated that the blood glucose in the STZ-induced diabetic group in comparison to the control group was significantly higher ($P<0.001$). Administration of 100 mg/kg and 200 mg/kg dose of *Biarum Bovei* for two weeks causes a significant reduction ($P<0.01$) in the BS in contrast to the diabetic group but 50 mg/kg, 400 mg/kg and 800 mg/kg of the *Biarum Bovei* extracts have no effect on the BS level (Figure 1).

The MDA mean in control, diabetic groups and the diabetic group receiving *Biarum Bovei* extract:

The MDA mean in control and diabetic groups after 14 days indicated that MDA in diabetic group has significantly increased ($P<0.001$) (Figures 2-5).

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Table 1. Effect of *Biarum Bovei* Extract (BBE) on lipid profiles, treated diabetic groups compared to untreated diabetic and control groups, Mean± SEM

Goups Lipid profiles (mg/dl)	Control	Untreated diabetic	Diabetic +100mg/kg BBE	Diabetic +200mg/kg BBE	Diabetic +400mg/kg BBE	Diabetic +800mg/kg BBE
TG	79.42± 10.7	167.14± 16 ***	117.2± 4.35 #	132± 3.8	100± 7.8 ###	116.4± 7.5 #
Chol	78.42± 3.61	112.86± 6.63 *	95.71± 14.45	101.57± 7.75	73.28± 2.44 ##	69371± 4.46 ##
LDL	10± 1.6	48.7± 6.1 ***	66.34± 2.3	30.8± 5.1	22.25± 1.25 ##	13.57± 7.9 ###
VLDL	15.8± 2.1	32.9± 3.1 ***	23.45± 0.8 #	26.4± 0.7	20.02± 1.56 ###	23.28± 1.51 #
HDL	52.71± 4.2	26.28± 2.6 ***	32.85± 2.1 #	31± 1.1 #	40± 3.1 ##	38.42± 2.04 ##

* Significant compared to the control
# Significant compared to the diabetic control
(One-way ANOVA-Post Hoc LSD test, n=7, $^{\#,*}P<0.05$, $^{\#\#,**}P<0.01$, $^{\#\#\#,***}P<0.001$)

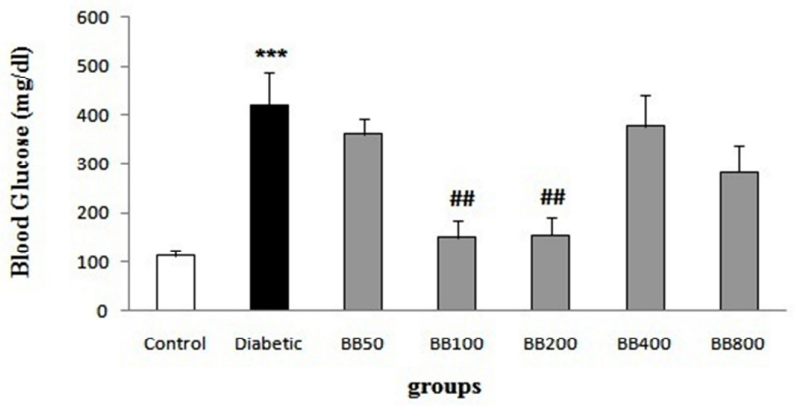

Figure 1. The comparison (Mean±standard deviation) of the BS level in control group with diabetes-induced group and the group receiving 50, 100, 200, 400 and 800 mg/kg extracts .
*** is suggestive of the significant difference from the control group and the sign # is indicative of the significant difference from the diabetic groups. (n=7, one-way variance analysis and Tukey back-up test)**

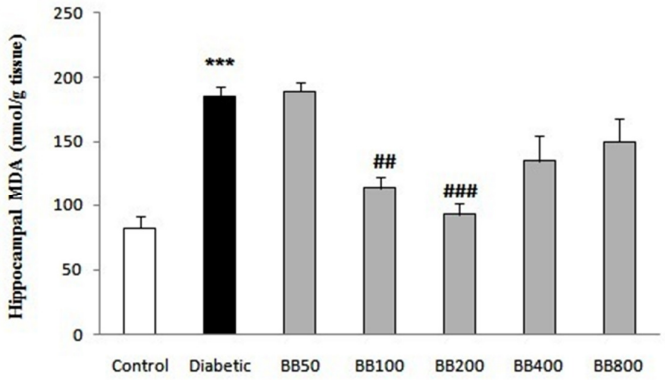

Figure 2. the comparison between MDA rate (mean±standard deviation) in hippocampus tissues of the control group with diabetic group and the diabetic group receiving a 50, 100, 200, 800 and 400 mg/kg *Biarum Bovei* (BB) extract dosage.
***is suggestive of the significant difference from the control group and # is indicative of the significant difference from the diabetic groups. (n=7, one-way variance analysis and Tukey back-up test)**

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In another part of the study pertaining to the MDA assay between the diabetic groups and the diabetic group receiving *Biarum Bovei* extract with the dose of 50, 100, 200, 800, 400 mg/kg for 14 consecutive days orally (Gavage method), it was shown that MDA rate decreased in the Hippocampus tissue of the groups receiving 100 mg/kg and 200 mg/kg with ($P<0.001$) and ($P<0.01$) significantly, (Figure 2). In the brain cortex tissue a significant decrease was also observed in the groups receiving 100 mg/kg and 200 mg/kg extract with ($P<0.01$) and ($P<0.001$), respectively (Figure 4). In striatum tissues the entire *Biarum Bovei* dose have been discovered to reduce MDA rate except 50 mg/kg dose (Figure 3), and it was also shown that the significance level has been ($P<0.01$) for 200 mg/kg dose and for the remaining dose as it has been found to be ($P<0.001$). In striatum tissues also only 100 mg/kg dosage reflected a significant decrease ($P<0.01$) (Figure 5).

Consumption of *Biarum Bovei for* two weeks significantly lowered levels of TG (100, 400,800 kg/mg, at $P<0.05$, $P<0.001$, $P<0.05$ respectively), cholesterol (400,800 kg/mg, $P<0.05$), LDL (400, 800 kg/mg, $P<0.01$ $P<0.001$ respectively) and VLDL(100, 400, 800 kg/mg, at $P<0.05$, $P<0.001$, $P<0.05$ respectively). Meanwhile, HDL increased (100, 200, 400,800 kg/mg, at $P<0.05$, $P<0.05$ $P<0.01$, $P<0.01$ respectively). (Table 1)

Discussion

The results of the current study indicated that the MDA rate in cerebral tissues and the blood glucose significantly increased. Consumption of various and numerous dose of the *Biarum Bovei* extracts enabled us to reduce the blood glucose and MDA levels.It also showed good effects on lipid profiles. Inducing diabetes mellitus by making use of STZ makes oxidative stress to occur in parts of the brain (19). It has been recently reported that the extremely over active oxidative stress plays a critical role in diabetic pathogenesis (20). The human body possesses its own defensive mechanism and this is the factor which brings about a complete control over the reactive oxygen species (ROS) plasma concentrations. But it has been revealed that in the individuals with type-2 diabetes, the increase in ROS plasma levels and the explicit decrease in the antioxidants lead to oxidative stress and this in turn will result in numerous detrimental effects in type-2 diabetes. Thus, it can be resulting from direct or indirect reduction in the oxidative stress (20). Lipid peroxidation is an important biological outcome indicative of the extremely severe oxidative dissolution of the cellular membrane and there are also reports implying the increase in lipid peroxidation level in the patients with diabetes (21). It indicated that the accumulation in the products resulting from the lipid peroxidation can cause oxidative stresses to the diabetic rats' cerebral tissues and blood vessels (19). Several researchers have shown that some herbs like Cassia auriculata and Ginger rhizome and Chenkadali cause a decrease in serum lipids (22,23). Favorable changes in lipid parameters such reduction of LDL, Colestrol, TG, and an increase in HDL attributed to flavonoids and phenolic compounds of extract (24). Phenolic compounds with strong antioxidant activity have been identified in edible members of Araceae family (25). Therefore antioxidants that are able to lower serum lipids reduce disease complications such as complications of cardiac-vascular diseases due to hyperlipidemia. Antioxidants are among the substances which act as barriers to the cellular damages through reducing the free radicals rates (2). To defend themselves against free radicals attacks, the cells create different antioxidant systems. There are antioxidant molecules with low molecular weight such as Uric Acid, Ascorbic Acid, Glutathione and so forth and the antioxidant enzymes such as Superoxide Dismutase, Catalase, and Glutathione Peroxidase. Under physiological conditions, such a defensive mechanism keeps the free radicals below a perpetually low level in the cells and their activities are precisely regulated (26). Traditionally, during the course

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of history, various plants have been used for reducing the blood sugar and improving the diabetes effects and in Iranian traditional medicine and in the other parts of the world, as well, there are more or less detailed information in this regard (27). Medicinal plants have been prescribed worldwide due to their lower costs and their greater effectiveness (27). Due to the fact that the plants are considered as a very important and rich source of antioxidants the researches are increasing in this study field. The plants which are rich in antioxidant ingredients can protect the cells from oxidative damages (28). Secondary metabolites derived from the plants such as totally phenolic and flavonoid compounds have been discovered to have strong potentials in clearing the free radicals and they are found everywhere in plants such as in the plants' leaves, fruit, seeds, roots and skins. Such a potential depends on the number of the aromatic loops and the nature of the hydroxyl dislocating groups (29). Flavonoids are polyphenols which have been found to have diverse biological activities such as inhibition of platelet aggregation, gathering the free radicals, improving NO performance and reducing LDL in the plasma (30). Antioxidants are among the substances that act as barriers to the cell damage through reducing the free radicals (2). In *Biarum Bovei*, is extracted various numbers of flavons c-glycosides (31). Flavonoids have antimicrobial, antioxidant and anti-platelets and anti-carcinogenic activities (32). Also, this group of the plants has been recognized with a characteristic of producing alkaloids, amins and flavonoids (13). Among the important uses of the *Biarum Bovei* extract we can refer to the treatment of the diseases such as blood lipids, hypertension, infection, diabetes and jaundice (33). In the current study, *Biarum Bovei* effects were shown on reducing lipid peroxidation for the first time which is an important oxidative stress factor in the diabetic animals' model. Therefore, it can be stated that the antioxidant compounds including the flavonoids existing in *Biarum Bovei* are likely to reduce the MDA production and blood glucose, as well through lowering the oxidative stress. On the other hand diabetes mellitus is closely associated with dyslipidemia. Due to very good antioxidant properties of *Biarum Bovei* Extract and beneficial effect on fat loss and antiatherogenic factor, presumably dose-dependently reduces the symptoms of diabetes and also decreases hazards and consequences of hyperlipidemia.

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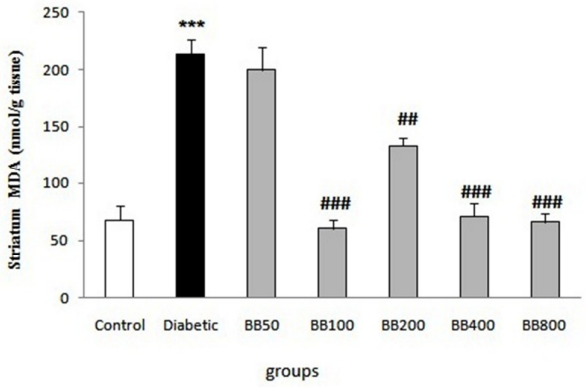

Figure 3. The comparison between MDA rate (mean ± standard deviation) in striatum tissues of the control group with diabetic group and the diabetic group receiving a 50, 100, 200, 800 and 400 mg/kg *Biarum Bovei* (BB) extract dose
*** is suggestive of the significant difference from the control group and # is indicative of the significant difference from the diabetic groups. (n=7, one-way variance analysis and Tukey back-up test)**

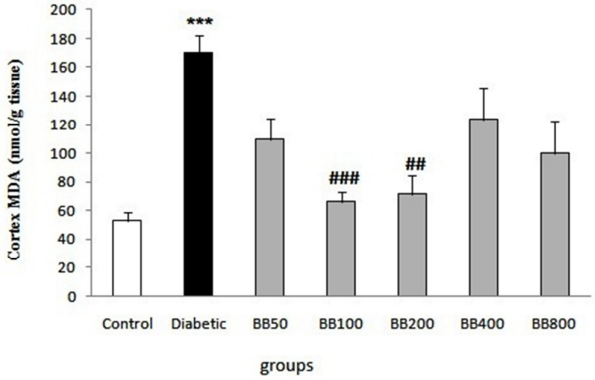

Figure 4. The comparison between MDA rate (mean ± standard deviation) in cortex tissues of the control group with diabetic group and the diabetic group receiving a 50, 100, 200, 800 and 400 mg/kg *Biarum Bovei* (BB) extract dosage
***is suggestive of the significant difference from the control group and # is indicative of the significant difference from the diabetic groups. (n=7, one-way variance analysis and Tukey back-up test)**

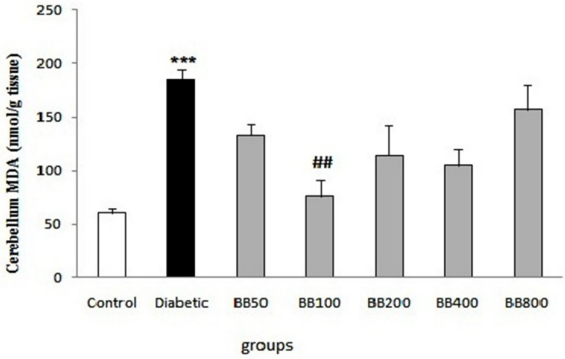

Figure 5. the comparison between MDA rate (mean ± standard deviation) in cerebellum tissues of the control group with diabetic group and the diabetic group receiving a 50, 100, 200, 800 and 400 mg/kg *Biarum Bovei* (BB) extract dosage
*** is suggestive of the significant difference from the control group and # is indicative of the significant difference from the diabetic groups. (n=7, one-way variance analysis and Tukey back-up test).**

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