

# Anti-hyperglycemic and anti-hyperlipidemic effects of *Vaccinium myrtillus* fruit in experimentally induced diabetes (antidiabetic effect of *Vaccinium myrtillus* fruit)

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## Abstract

**BACKGROUND:** Diabetes mellitus (DM) is a metabolic disorder that is associated with an increased risk of cardiovascular disease. *Vaccinium myrtillus* (bilberry) is a useful plant with antidiabetic properties in traditional medicine. The aim of this study was to investigate the effects of bilberry against DM. Diabetes was induced using intraperitoneal injection of alloxan (120 mg kg<sup>-1</sup> body weight (BW)). Bilberry powder (2 g d<sup>-1</sup>) and glibenclamide (positive control; 0.6 mg kg<sup>-1</sup> BW) were administered for 4 weeks following alloxan injection. Serum glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very-low-density lipoprotein cholesterol (VLDL-C), triglycerides (TG) and C-reactive protein (CRP) were determined at baseline and at 2nd and 4th week of the study.

**RESULTS:** Bilberry supplementation resulted in a significant reduction of glucose compared with the diabetic control as well as glibenclamide treatment. Bilberry elevated insulin, reduced TC, LDL-C, VLDL-C and TG levels, and prevented HDL-C decline. Serum insulin, TC and LDL-C levels were not affected by glibenclamide, and CRP did not significantly change with either bilberry or glibenclamide. Histological examinations revealed a significant elevation of islet size in the bilberry and glibenclamide-treated groups.

**CONCLUSION:** Dietary supplementation with bilberry fruits may protect against impaired glucose and lipid metabolism in DM.

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**Keywords:** *Vaccinium myrtillus*; diabetes mellitus; dyslipidemia; glibenclamide; alloxan

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by insulin resistance and disturbed lipid and glucose metabolism, which results in several macro- and microvascular complications.<sup>1</sup> Insulin resistance drives visceral free fatty acid flux to the liver, leading to increased hepatic assembly and secretion of very-low-density lipoproteins (VLDLs), in particular the larger VLDL<sub>1</sub> subfraction.<sup>2,3</sup> Lipid abnormalities in type 2 DM is characterized by elevated serum triglycerides, reduced concentrations of high-density lipoproteins (HDLs) and increased formation of small, dense, low-density lipoprotein (sdLDL) particles.<sup>4</sup> Triglyceride-rich lipoproteins are substrates of the hepatic lipase and transformed into sdLDL after lipase-mediated triglyceride hydrolysis.<sup>5</sup> Correction of hyperglycemia and hyperlipidemia are integral to the prevention of vascular complications in diabetic patients. In recent decades there has been interest in using alternative therapies for the management of DM. Several plants have been used in traditional medicine to prevent diabetes and mitigate its complications.<sup>6</sup>

*Vaccinium myrtillus* (bilberry) is a medicinal plant belonging to the Ericaceae family. It is found in the mountains and forests of Europe and the northern USA.<sup>7</sup> Fruits of this plant have been

used in traditional medicine for the treatment of various diseases, including fevers, coughs, renal stones, intestinal and liver disorders and stomatitis.<sup>8</sup> Pharmacological studies have shown that bilberry fruit is an effective treatment for ocular disorders such as retinopathy and vascular disorders.<sup>7</sup> There is also evidence for the antidiabetic and hypoglycemic activity of bilberry fruits.<sup>9</sup> Bilberry fruits are rich in bioactive phytochemicals such as anthocyanins, quercetin and catechins, tannins, vitamins and pectins.<sup>10</sup> Total content of phenolic acids in bilberry fruits is 31.7 ± 0.54 mg 100 g<sup>-1</sup> fresh weight, and mainly include quercetin, myricetin,

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cafeic acid and *p*-coumaric acid.<sup>11</sup> These phytochemicals are known to possess antioxidant and antidiabetic actions, making bilberry fruit a potential supplement for DM.<sup>7</sup> Given the strong evidence supporting biological activities of bilberry phytochemicals, the present study aimed to investigate the anti-hyperglycemic and anti-hyperlipidemic activities of bilberry fruit in an experimentally induced diabetic rat model.

## MATERIALS AND METHODS

### Measurement of anthocyanin content:

Total anthocyanin content of bilberry fruit was determined using the pH differential method, as previously described.<sup>12</sup>

### Plant preparation

Fruits of *V. myrtillus* were collected in September from Arasbaran, one of the northern cities of Iran, and identified at the Medical Plants Research Center of Shahrekord (voucher no. 202). Fruits were washed and dried in shade at room temperature (20–22 °C) for 2 weeks.

### Experimental design

A total of 32 adult male Wistar rats weighing about 190–240 g were housed in cages at 24 °C, with a 12 h light/dark cycle, and were given a standard rat chow diet. The experimental protocol was approved by the Ethics Committee of the Isfahan University of Medical Sciences (Isfahan, Iran) and was according to the approved standards for laboratory animal care.

The animals were allowed 2 weeks for acclimatization. Alloxan (Sigma Aldrich, USA) was administered intraperitoneally at a single dose of 120 mg kg<sup>-1</sup> body weight (BW) to induce diabetes. Induction of diabetes was confirmed by measuring fasting plasma glucose levels after 72 h of alloxan injection. Rats with plasma glucose levels > 250 mg dL<sup>-1</sup> were considered as diabetic and used for the experiments. Animals were randomly divided into four equal groups (*n* = 8) as follows: 1 – non-diabetic control rats that were given a basic diet; 2 – alloxan-induced diabetic control rats given a basic diet; 3 – alloxan-induced diabetic rats receiving glibenclamide intraperitoneally at a dose of 0.6 mg kg<sup>-1</sup> BW (Hakim Pharmacy, Iran) with basic diet; and 4 – alloxan-induced diabetic rats receiving bilberry powder at a dose of 2 g d<sup>-1</sup> (via gavage) with basic diet.<sup>13</sup> Animals in all groups were treated for 4 weeks. At the start of experiments and at the end of the 2nd and 4th week of study, blood samples were collected into EDTA (ethylenediaminetetraacetic acid) tubes and plasma was isolated by centrifugation. After the last blood sampling, animals were sacrificed and each pancreas was removed and fixed in 10% formalin. Sections were cut and stained by hematoxylin and eosin (H&E) for histological examination.

### Biochemical analyses

Serum glucose was determined by the glucose oxidase method using a commercial enzymatic kit (Pars azmoon, Iran). Serum insulin levels were determined using an enzyme-linked immunosorbent assay (ELISA) method (Monobind Inc., USA). C-reactive protein (CRP) was measured in plasma via ELISA (Diagnostics Biochem Com/ Canada). Lipid profile comprising total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides was determined using routine enzymatic methods with commercial kits on an autoanalyzer (Hitachi Co., Tokyo, Japan).

VLDL-cholesterol (VLDL-C) levels were calculated using Friedewald's formula.<sup>14</sup>

### Statistical analysis

Data expressed as mean ± SE and analyzed using the Statistical Package for the Social Sciences (SPSS) software program (version 15, SPSS Inc., Chicago, IL, USA). The significance level of *P* < 0.05 was considered for all the analyses. The Kruskal–Wallis test was used to compare between effects based on time. For multiple comparison the Mann–Whitney test with Bonferroni correction was used. To determine the time effect based on each group the Fridmann test was used. Repeated-measures analysis of variance (RMANOVA) was used to determine the interaction effect of time and group.

## RESULTS

The ripe bilberry fruits contained 15.5% protein, 30.6% carbohydrates, 1.5% total fat, 2% soluble solids, 22.3% nitrogen and 1.4% calcium. Each 500 mg of bilberry fruit powder contained 45 ± 2 mg of anthocyanins.

Alloxan treatment caused a significant elevation of blood glucose, whereas blood glucose remained similar to baseline levels in the non-diabetic group. Supplementation for 2 and 4 weeks with bilberry as well as with glibenclamide resulted in a significant reduction of fasting blood glucose compared with the diabetic control group (Table 1).

Serum insulin levels were significantly reduced in the diabetic group. In contrast, insulin levels in the group receiving glibenclamide remained statistically unchanged by the end of the study. Supplementation with bilberry significantly elevated serum insulin compared with the control diabetic rats (Table 1). Alloxan treatment had an increasing effect on serum total cholesterol, LDL-C, triglycerides and VLDL-C concentrations, but all of these parameters were significantly reduced following supplementation with bilberry powder. In the glibenclamide-treated group, VLDL-C and triglycerides were the only parameters that were reduced. Serum HDL-C concentrations were reduced in the diabetic control group compared to the non-diabetic group, while there was no change in the groups receiving glibenclamide or bilberry, as also observed in the non-diabetic control group. Serum CRP concentrations did not significantly change in any of the study groups (Table 1).

Histological examination of pancreatic tissues indicated increased size of Langerhans islets following treatment with bilberry and glibenclamide compared with the diabetic control group (Table 2 and Fig. 1).

## DISCUSSION

The present study indicated a positive impact of supplementation with bilberry on metabolic abnormalities induced by alloxan. Bilberry fruit supplementation could reverse the hyperglycemic and hyperlipidemic effects of alloxan.

Previous studies have shown that bilberry lowers blood glucose in both animals<sup>15,16</sup> and humans,<sup>17</sup> and is useful for the prevention and treatment of diabetic retinopathy.<sup>8</sup>

The chemical composition of *Vaccinium* species includes anthocyanins, flavonoids, coumarins, sterols, triterpenoids, pectins and alkaloids.<sup>18,19</sup> Anthocyanins, in particular myrtillin anthocyanoside, are some of the most active hypoglycemic constituents

**Table 1.** Average levels of biochemical parameters in non-diabetic control, diabetic control, diabetic rats treated with bilberry powder and diabetic rats treated with glibenclamide (mean  $\pm$  SEM;  $P < 0.05$ )

Biochemical factor	Time	Non-diabetic (n = 8)	Diabetic (n = 8)	Glibenclamide (n = 8)	Bilberry (n = 8)	p-value
Fasting blood glucose (mg dL <sup>-1</sup> )	1	56.00 $\pm$ 16.17	48.80 $\pm$ 2.38	55.50 $\pm$ 4.23	47.57 $\pm$ 6.57	0.200
	2	73.20 $\pm$ 10.42	291.00 $\pm$ 70.37*	81.33 $\pm$ 31.40 <sup>†</sup>	74.22 $\pm$ 29.17 <sup>†</sup>	0.008
	3	69.71 $\pm$ 16.44	316.40 $\pm$ 66.56* <sup>‡</sup>	95.500 $\pm$ 21.09 <sup>†</sup>	83.71 $\pm$ 79.19 <sup>†</sup>	0.002
	p-value	0.076	0.015	0.074	0.054	
Insulin ( $\mu$ g dL <sup>-1</sup> )	1	2.14 $\pm$ 0.66	1.98 $\pm$ 0.84	2.30 $\pm$ 0.44	1.96 $\pm$ 0.32	0.120
	2	1.92 $\pm$ 0.82	1.21 $\pm$ 0.23	2.25 $\pm$ 1.41	2.11 $\pm$ 0.26	0.078
	3	2.23 $\pm$ 0.71	0.83 $\pm$ 0.80* <sup>‡</sup>	2.00 $\pm$ 0.05	2.41 $\pm$ 0.33 <sup>†</sup>	0.048
	p-value	0.281	0.049	0.673	0.875	
LDL-cholesterol (mg dL <sup>-1</sup> )	1	17.00 $\pm$ 1.41	16.80 $\pm$ 2.28	19.40 $\pm$ 4.50	16.83 $\pm$ 1.94	0.084
	2	19.25 $\pm$ 5.37	20.60 $\pm$ 3.04	19.60 $\pm$ 3.84	18.33 $\pm$ 3.77	0.751
	3	18.14 $\pm$ 2.73	25.20 $\pm$ 1.64* <sup>‡</sup>	21.20 $\pm$ 4.65	17.22 $\pm$ 2.72 <sup>†</sup>	0.007
	p-value	0.264	0.028	0.607	0.867	
HDL-cholesterol (mg dL <sup>-1</sup> )	1	54.40 $\pm$ 5.94	60.25 $\pm$ 2.21	59.33 $\pm$ 6.34	52.50 $\pm$ 10.78	0.349
	2	51.00 $\pm$ 2.16	52.60 $\pm$ 3.43	60.50 $\pm$ 9.39	54.12 $\pm$ 8.96	0.218
	3	51.50 $\pm$ 3.27	34.80 $\pm$ 4.49* <sup>‡</sup>	51.66 $\pm$ 10.30	44.85 $\pm$ 9.58	0.009
	p-value	0.472	0.018	0.070	0.264	
Total cholesterol (mg dL <sup>-1</sup> )	1	105.40 $\pm$ 11.17	107.80 $\pm$ 13.47	111.66 $\pm$ 18.31	98.60 $\pm$ 9.56	0.191
	2	95.00 $\pm$ 14.30	107.75 $\pm$ 11.67	111.83 $\pm$ 22.99	96.33 $\pm$ 31.98	0.433
	3	92.28 $\pm$ 7.29	136.20 $\pm$ 23.49* <sup>‡</sup>	94.83 $\pm$ 23.03	81.33 $\pm$ 21.34 <sup>†</sup>	0.009
	p-value	0.105	0.045	0.115	0.264	
Triglyceride (mg dL <sup>-1</sup> )	1	110.00 $\pm$ 29.81	103.80 $\pm$ 16.78	94.50 $\pm$ 23.33	93.20 $\pm$ 20.58	0.617
	2	83.60 $\pm$ 13.63	113.40 $\pm$ 59.18	83.33 $\pm$ 5.92	84.11 $\pm$ 42.40	0.920
	3	97.14 $\pm$ 17.9	149.60 $\pm$ 19.13* <sup>‡</sup>	47.66 $\pm$ 14.17 <sup>†‡</sup>	49.12 $\pm$ 21.39 <sup>†‡</sup>	0.003
	p-value	0.022	0.015	0.009	0.047	
VLDL-cholesterol (mg dL <sup>-1</sup> )	1	19.00 $\pm$ 2.00	20.76 $\pm$ 3.35	18.90 $\pm$ 4.66	18.64 $\pm$ 4.11	0.755
	2	16.72 $\pm$ 2.72	22.68 $\pm$ 11.83	16.50 $\pm$ 1.29	16.75 $\pm$ 8.40	0.906
	3	13.34 $\pm$ 3.52	29.92 $\pm$ 3.82* <sup>‡</sup>	9.53 $\pm$ 2.83 <sup>†‡</sup>	9.82 $\pm$ 4.27 <sup>†‡</sup>	0.003
	p-value	0.060	0.045	0.009	0.037	
C-reactive protein (mg dL <sup>-1</sup> )	1	42.40 $\pm$ 4.92	41.40 $\pm$ 2.190	44.50 $\pm$ 4.54	43.00 $\pm$ 2.19	0.568
	2	49.75 $\pm$ 9.21	47.80 $\pm$ 8.49	45.50 $\pm$ 7.84	45.57 $\pm$ 5.62	0.707
	3	41.14 $\pm$ 7.58	54.40 $\pm$ 4.77	50.33 $\pm$ 3.50	43.37 $\pm$ 7.34	0.013
	p-value	0.174	0.074	0.260	0.627	

Time 1, baseline; time 2: 2 weeks after alloxan injection; time 3, 4 weeks after alloxan injection.

 \*Indicates a significant difference with non-diabetic control group; <sup>†</sup>indicates a significant difference with diabetic group; <sup>‡</sup>indicates a significant difference between times 1 and 3.

**Table 2.** Average size of pancreatic islets in bilberry and glibenclamide diabetic treated rats (mean  $\pm$  SEM) ( $P < 0.05$ )

Group	Pancreas islet size ( $\mu$ )
Non-diabetic (n = 8)	1.21 $\pm$ 0.18
Diabetic (n = 8)	0.56 $\pm$ 0.07*
Glibenclamide (n = 8)	1.05 $\pm$ 0.25 <sup>†</sup>
<i>Vaccinium</i> (n = 8)	1.07 $\pm$ 0.39 <sup>†</sup>
P-value	0.005

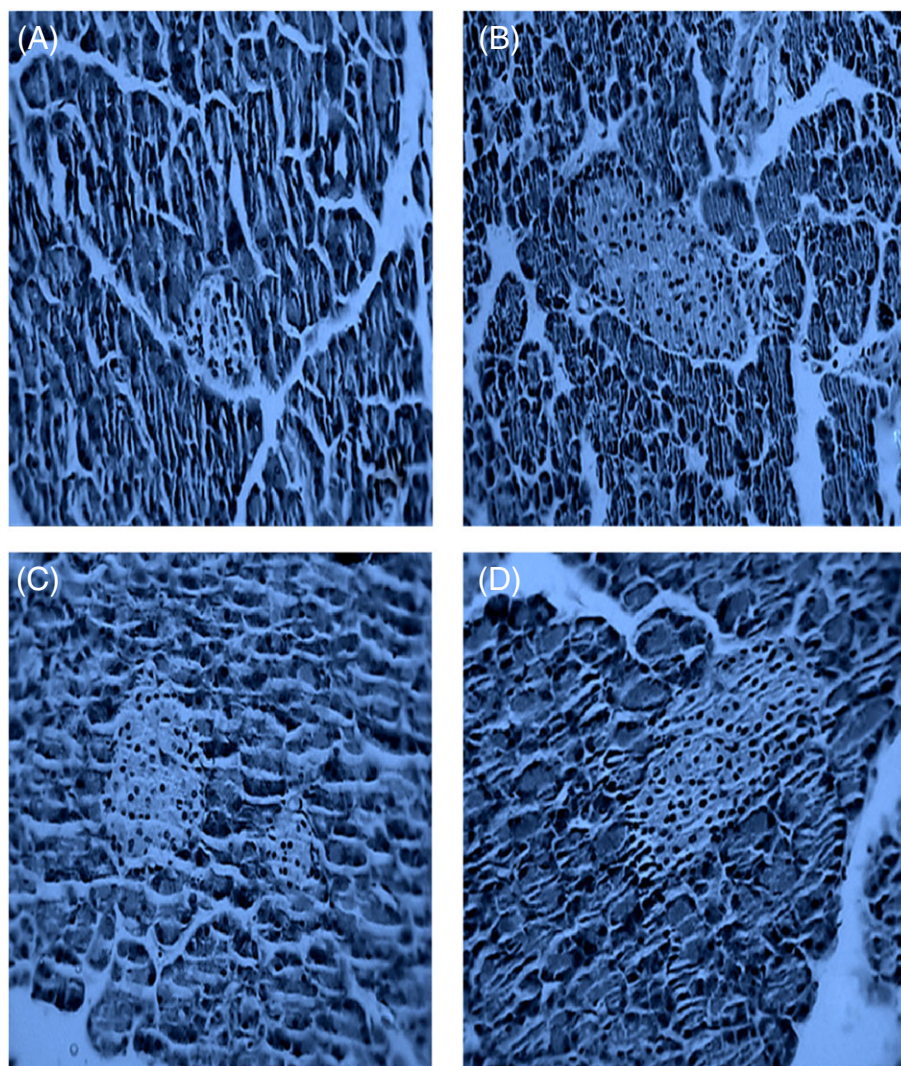
 \*Means differ significantly from non-diabetic control group <sup>†</sup>indicates a significant difference from diabetic group.

among other components of *Vaccinium* species.<sup>15</sup> In addition, numerous health benefits have been identified for these phytochemicals, including antioxidant, anti-inflammatory, cardioprotective and vision-improving activities.<sup>19</sup> Previous investigations have shown that bilberry fruit containing anthocyanins is useful for the prevention and treatment of type 2 diabetes.<sup>18,20</sup>

In the present study, bilberry supplementation elevated serum insulin levels in alloxan-induced diabetic rats. It has been reported that anthocyanin stimulates insulin secretion in rodent pancreatic  $\beta$  cells in the presence of glucose, and attenuates obesity and insulin resistance in mice.<sup>20,21</sup> In addition, anthocyanins have  $\alpha$ -glucosidase inhibitory activity and can inhibit intestinal absorption of glucose.<sup>22,23</sup> Flavonoids have been identified as antidiabetic components: Nica *et al.* demonstrated the role of flavonoids in mediating the metabolic effects of insulin.<sup>24</sup> In addition, it has been reported that oleanolic acid, a triterpenoid found in the *Vaccinium* genus, increases the release of acetylcholine from nerve terminals. Acetylcholine stimulates muscarinic M3 receptors in pancreatic cells and increases the release of insulin.<sup>25</sup> Thus oleanolic acid is one of the active constituents likely to be responsible for the observed increases in serum insulin concentrations following bilberry supplementation.<sup>25,26</sup>

In the current study, bilberry not only improved hyperglycemia but also significantly attenuated diabetic dyslipidemia by reducing plasma levels of total cholesterol, LDL-C, VLDL-C and triglycerides. A previous study has indicated the beneficial effects of *Vaccinium* treatment on serum lipid profile and oxidative stress





**Figure 1.** Section of Langerhans islets, H&E staining (40 $\times$ ): (A) diabetic; (B) non-diabetic; (C) treated with glibenclamide; (D) treated with bilberry.

in hyperlipidemic adult patients.<sup>27</sup> Anthocyanins may account for the lipid-modifying effects of bilberry, which act through regulating the activity of enzymes involved in lipoprotein metabolism.<sup>21,28</sup> Moreover, fibers such as pectin that are present in bilberry possess hypolipidemic activity owing to their inhibitory effect on intestinal absorption of cholesterol and bile acids.<sup>29</sup> The plant also contains phytosterols, which are structurally similar to cholesterol and competitively inhibit intestinal absorption of cholesterol.<sup>30</sup>

One of the potential clinical benefits of bilberry is its anti-inflammatory effect. Bioflavonoids and anthocyanosides present in bilberry prevent the release and synthesis of pro-inflammatory compounds such as histamine, prostaglandins and leukotrienes.<sup>31</sup> Oleonic acid has also been shown to have anti-inflammatory and hepatoprotective effects.<sup>25</sup> In the present study, serum CRP levels did not change in any of the study groups; however, this might be due to the lack of significant effect of alloxan-induced diabetes on the development of systemic inflammation, as serum CRP was comparable between non-diabetic and diabetic rats at all assessed time points of study, i.e. baseline and weeks 2 and 4.

A significant improvement was observed in the histological findings of pancreas tissue following supplementation with bilberry. Plant antioxidants such as alkaloid, terpenoids and sterol have

been shown in previous studies to restore and regenerate pancreatic  $\beta$  cells and reduce tissue inflammation in experimental models of diabetes.<sup>32,33</sup> As mentioned earlier, bilberry contains several antioxidant components that can protect against oxidative stress-induced pancreatic damage via attenuation of membrane lipid peroxidation and blunting other detrimental effects of free radicals.<sup>29</sup> In addition, the anti-inflammatory properties of bilberry can prevent pancreatic damage induced by alloxan.<sup>19,31</sup>

## CONCLUSIONS

To sum up, findings of the present experimental study indicated the anti-hyperglycemic and anti-hyperlipidemic efficacy of bilberry fruit in alloxan-induced diabetic rats. It is interesting to note that the lipid-modifying effects of bilberry included correction of cholesterol and triglyceride levels, an effect that is particularly important for the treatment of mixed dyslipidemias. Another important aspect of the present study is the use of crude fruit without any extraction or processing. Hence, even greater antidiabetic and lipid-modifying effects are expected with the use of bilberry extracts concentrated with the bioactive phytochemicals of the plant. Further studies are recommended to explore the

protective effects of bilberry against diet-induced hyperlipidemia. Finally, given the strong ethno-medicinal background and safety of bilberry fruit, clinical trials are warranted to evaluate the benefits of dietary supplementation with bilberry in patients with cardiometabolic disorders.

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