

ANTIDIABETIC AND HYPOGLYCEMIC ACTIVITY OF PROTEIN ISOLATE FROM *BERBERIS VULGARIS* FRUITS

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Abstract. This study investigates the antidiabetic and hypoglycemic activity of a protein isolate obtained from *Berberis vulgaris* fruits (BPI). The isolate was prepared using acid extraction followed by isoelectric precipitation, yielding 3.84% with a protein content of 44.1% as determined by the Lowry method. SDS-PAGE analysis revealed a heterogeneous distribution of proteins with molecular weights ranging from 15 to 70 kDa, indicating the presence of both low- and high-molecular-weight fractions, including potential bioactive peptides. The biological activity of BPI was evaluated in an alloxan-induced diabetes model in rats. Oral administration of BPI at a dose of 25 mg/kg for 14 days resulted in a significant reduction in blood glucose levels compared to the control group, along with stabilization of body weight. By day 14, glucose levels decreased to 5.9 ± 0.3 mmol/L versus 11.2 ± 0.43 mmol/L in untreated diabetic animals. The observed hypoglycemic effect may be associated with the presence of bioactive peptides capable of inhibiting carbohydrate-digesting enzymes, improving insulin sensitivity, and reducing oxidative stress. Overall, the findings demonstrate that BPI exhibits pronounced antidiabetic potential and may serve as a promising natural source for the development of functional foods and nutraceuticals aimed at diabetes management.

Keywords: *berberis vulgaris*; barberry protein isolate; antidiabetic activity; hypoglycemic effect; alloxan-induced diabetes; bioactive peptides; blood glucose regulation.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes continues to rise, posing a significant burden on healthcare systems and increasing the risk of severe complications such as cardiovascular diseases, neuropathy, nephropathy, and retinopathy. Although synthetic antidiabetic drugs are widely used, their long-term administration is often associated with adverse effects, high costs, and limited accessibility in some regions. Therefore, the search for safer, more effective, and naturally derived therapeutic agents remains an important area of research.

Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse pharmacological properties, including antidiabetic and hypoglycemic effects. Among them, *Berberis vulgaris* (barberry) has attracted considerable attention due to its rich phytochemical composition and traditional use in managing metabolic disorders. The fruits of this plant are known to contain alkaloids, phenolic compounds, organic acids, vitamins, and other biologically active substances that contribute to its therapeutic potential [1-3].

While most studies have focused on low-molecular-weight compounds such as berberine, recent attention has shifted toward macromolecules, particularly proteins and bioactive peptides, which may exhibit significant biological activities. Protein isolates derived from plant sources are

increasingly recognized for their potential health benefits, including antioxidant, anti-inflammatory, and glucose-regulating effects. However, the antidiabetic potential of protein isolates obtained from *Berberis vulgaris* fruits remains insufficiently explored. Protein isolates may exert hypoglycemic effects through various mechanisms, such as inhibition of carbohydrate-digesting enzymes, enhancement of insulin sensitivity, modulation of glucose uptake, and protection of pancreatic β -cells from oxidative stress [4,5]. Investigating these mechanisms could provide new insights into the development of functional foods or nutraceuticals aimed at diabetes management.

The aim of study is to investigate the antidiabetic and hypoglycemic activity of a protein isolate obtained from the fruits of *Berberis vulgaris*. The study seeks to evaluate its potential effects on glucose regulation and to explore its possible mechanisms of action, including its influence on carbohydrate metabolism and insulin-related pathways. Furthermore, this research aims to assess the feasibility of using this plant-derived protein isolate as a natural therapeutic agent for the management of diabetes mellitus.

Materials and methods

Preparation of Barberry Protein Isolate (BPI). Protein isolates were prepared from freeze-dried barberry fruits using acid extraction according to a modified method of Acosta et al. [6]. Briefly, 100 g of freeze-dried fruit powder was mixed with 1 L of 10% (v/v) acetic acid solution (pH $\sim$ 2.3) in a glass beaker and stirred continuously using a magnetic stirrer at room temperature ($22 \pm 2^\circ\text{C}$) for 3 hours to facilitate protein solubilization. The mixture was then centrifuged at 6000 rpm ($5000 \times g$) for 20 minutes at 4°C using a refrigerated centrifuge (Eppendorf 5810 R, Germany), and the supernatant was collected. The pellet was re-extracted with 500 mL of 10% acetic acid for 1 hour, centrifuged, and the supernatants were combined. Protein precipitation was achieved by adjusting the pH of the combined supernatant to 4.5 (the isoelectric point of most plant proteins) using 1 M NaOH solution with continuous stirring. The precipitate was allowed to settle overnight at 4°C , then collected by centrifugation at 6000 rpm for 20 minutes. The protein precipitate was washed three times with distilled water (pH adjusted to 4.5) to remove residual salts and acetic acid, then freeze-dried at -55°C and 0.05 mbar for 48 hours. The dried BPI was ground to a fine powder, stored in airtight containers at -20°C , and protected from light until analysis. The yield of protein isolate was calculated as:

$$\text{Yield (\%)} = (\text{Weight of BPI} / \text{Weight of freeze-dried fruit powder}) \times 100$$

Protein Content Determination. The protein content of BPI was determined using the Lowry method [7] with bovine serum albumin (BSA, Sigma-Aldrich, USA) as the standard. Briefly, BPI samples (1 mg) were dissolved in 1 mL of 0.1 M NaOH, and 0.1 mL of the solution was mixed with 1 mL of alkaline copper reagent (prepared by mixing 50 mL of 2% Na_2CO_3 in 0.1 M NaOH with 0.5 mL of 1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.5 mL of 2% sodium potassium tartrate). After 10 minutes of incubation at room temperature, 0.1 mL of Folin-Ciocalteu reagent (diluted 1:1 with distilled water) was added, and the mixture was vortexed immediately. After 30 minutes of incubation in the dark, absorbance was measured at 750 nm using a UV-Vis spectrophotometer (METASH UV-5100, China). A calibration curve was prepared using BSA solutions (0–1000 $\mu\text{g/mL}$), and results were expressed as μg protein per mg of BPI.

SDS-PAGE Analysis. SDS-PAGE was performed according to the Laemmli method to determine the molecular weight distribution of BPI proteins. Samples (2 mg/mL) were mixed with sample buffer (62.5 mM Tris-HCl, pH 6.8; 2% SDS; 10% glycerol; 5% β -mercaptoethanol; 0.01% bromophenol blue), heated at 95°C for 5 min, and cooled on ice. Electrophoresis was carried out

using a Dual Mini Vertical Electrophoresis Unit (Sigma-Aldrich, USA) with 12% resolving and 4% stacking gels at 120 V for ~90 min in Tris–glycine–SDS buffer. A pre-stained protein marker (10–180 kDa) was used as a reference. Gels were stained with Coomassie Brilliant Blue R-250 and destained until clear bands appeared. Protein molecular weights were estimated using standard markers (10–130 kDa).

Determination of antidiabetic and hypoglycemic activity. The experiments were conducted on 18 adult white rats weighing 220–250 g, maintained on a standard diet. The animals were divided into three groups, each consisting of 6 rats. In the first group, the studied parameters were evaluated under conditions of diabetes mellitus: pathological control (PC) — animals with experimentally induced diabetes receiving physiological saline with alloxan hydrate. The second group consisted of diabetic animals treated with the reference drug AlaDiabet (capsules, manufacturer: "AS-SUNNA PHARMACEUTICAL", Uzbekistan) at a dose of 106 mg/kg. The third group (PC) included diabetic animals treated with *Berberis vulgaris* protein isolate at a dose of 25 mg/kg. In this study, diabetes mellitus was induced by intraperitoneal injection of Alloxan at a dose of 120 mg/kg. The development of diabetes was monitored by an increase in blood glucose levels to no less than 17–20 mmol/L, as well as by increased water intake and body weight loss. *Berberis vulgaris* protein isolate was administered orally to animals with alloxan-induced diabetes once daily for 14 days at a dose of 25 mg/kg. The selected dose and duration of treatment were based on previously observed efficacy at this dosage. Animals were monitored for general condition over a two-week period under vivarium conditions. On the second day after diabetes induction, the *Berberis vulgaris* protein isolate substance was administered intragastrically at a dose of 25 mg/kg for 14 days. Blood samples were collected on day 7 to determine glucose levels. Blood glucose levels were measured using test strips and the Contour Plus glucometer.

Animal Ethics. All preoperative and experimental protocols have been thoroughly reviewed and approved by the Institutional Committee for the Use and Care of Animals. The animals were kept in the rooms of the vivarium under controlled conditions, with a relative humidity of 55-65%, an ambient temperature of 22 ± 2 °C and had free access to water and regular laboratory feed. All procedures for the treatment and care of animals strictly comply with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The ethical approval of this study was received by the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, the Committee on Animal Ethics (Protocol No. 133/1a/h dated August 4, 2014).

Results

The yield of barberry protein isolate (BPI) obtained using 10% acetic acid extraction was 3.84% of the initial freeze-dried fruit powder mass. This yield is comparable to protein isolates obtained from other fruit sources using similar acid extraction methods. For example, Acosta et al. [6] reported protein isolate yields of 4.2% from *B. microphylla* fruits using alkaline extraction followed by isoelectric precipitation. The relatively modest yield reflects the low total protein content of barberry fruits (10.7% DW) and the selectivity of the acid extraction method, which preferentially solubilizes certain protein fractions while leaving others in the insoluble residue [6, 8]. The protein content of BPI, determined by the Lowry method, was 441 µg/mg (44.1% w/w), indicating a high degree of protein enrichment in the isolate compared to the whole fruit (10.7% DW). The SDS-PAGE analysis of BPI (Figure) revealed protein bands at 15, 25, 35, 50, and 70 kDa, indicating a heterogeneous mixture of low-, medium-, and high-molecular-weight proteins obtained via acid extraction. The presence of low-molecular-weight fractions may reflect bioactive

peptides or partial protein hydrolysis. This compositional diversity may be linked to the antidiabetic potential of BPI, as such peptides are known to inhibit α -amylase and α -glucosidase, improve insulin sensitivity, and exert antioxidant effects [9-11] Additionally, higher-molecular-weight proteins may contribute synergistically or serve as precursors of bioactive peptides [12].

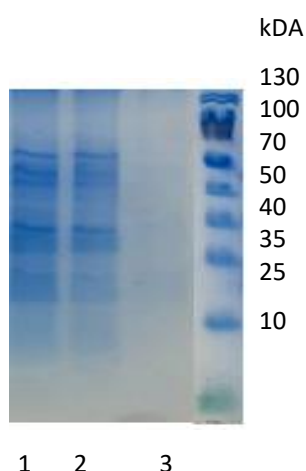


Figure. The electrophoretic profiles of *Berberis vulgaris* fruit protein isolate on SDS-PAGE; 1, 2: BPI, 2: Marker.

Table 1. Blood glucose levels in rats with alloxan-induced diabetes on days 7 and 14 after the administration of *Berberis vulgaris*, ($M \pm m$, $n=6$)

Experimental groups and doses	Initial values	Values on day 7 of the experiment		Values on day 14 of the experiment	
		Animal weight	Glucose, mmol/L	Animal weight	Glucose, mmol/L
Control, water 2 ml	301,6 $\pm$ 9,3	269 $\pm$ 2,3	9,6 $\pm$ 2,1	251,3 $\pm$ 6,0	11,2 $\pm$ 0,43
<i>AlaDiabet</i> 106 mg/kg	276,3 $\pm$ 8,6	287,3 $\pm$ 8,6	8,6 $\pm$ 2,7	297,2 $\pm$ 4,0** p=0.0001	8,9 $\pm$ 3,3
BPI, 25 mg/kg	299,1 $\pm$ 8,6	315,3 $\pm$ 13,7* p=0.008	7,3 $\pm$ 1,5	318,6 $\pm$ 7,3** p=0.00005	5,9 $\pm$ 0,3** p=0.000003

*p<0,01; **p<0,005 - significant differences versus the control group

The results showed a positive effect of BPI on metabolic parameters in experimental animals (Table 1). In the BPI-treated group, a stable increase in body weight was observed over 7 and 14 days, accompanied by a significant reduction in blood glucose levels. In particular, by day 7, the dynamics of body weight were significantly higher compared with the control group ($p<0.01$). After 14 days of treatment, body weight showed a statistically significant increase in both the *AlaDiabet* and BPI groups compared with the control group ($p<0.005$ for both groups). During the same period, a statistically significant decrease in blood glucose levels was observed in the BPI-treated group compared with the control group ($p<0.005$).

Although the reference drug *AlaDiabet* also demonstrated a certain degree of activity, the BPI-treated group exhibited more stable and pronounced antidiabetic effects. These findings suggest that the bioactive protein fractions and peptides present in BPI may play an important role in regulating glucose metabolism. Overall, these data indicate the potential of *Berberis vulgaris* proteins as a promising component of functional foods and nutraceuticals for the prevention and correction of metabolic disorders.

Conclusions. The results of this study demonstrate that the protein isolate obtained from *Berberis vulgaris* fruits possesses significant antidiabetic and hypoglycemic activity in an alloxan-

induced diabetes model. Oral administration of BPI at a dose of 25 mg/kg for 14 days led to a pronounced reduction in blood glucose levels and stabilization of body weight in experimental animals, indicating an improvement in metabolic status. SDS-PAGE analysis revealed a heterogeneous composition of proteins with molecular weights ranging from 15 to 70 kDa, suggesting the presence of both high-molecular-weight proteins and low-molecular-weight peptides. These bioactive fractions may play a key role in the observed biological effects, potentially through mechanisms such as modulation of carbohydrate metabolism, enhancement of insulin sensitivity, and reduction of oxidative stress.

Overall, the findings indicate that BPI represents a promising natural source of biologically active compounds with antidiabetic potential. The study supports the feasibility of using *Berberis vulgaris*-derived protein isolates as functional ingredients in the development of nutraceuticals or therapeutic agents for the management of diabetes mellitus.

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