INHIBITORY EFFECTS OF *CRATAEGUS CURVISEPALA*, SALVIA HYDRANGEA, AND *BETULA PENDULA* ON IN-VITRO PROTEIN GLYCOSYLATION

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Abstract

INTRODUCTION: Diabetes is one of the most common endocrine diseases spreading rapidly in the world. Diabetes complications are classified into acute and chronic. Non-enzymatic glycosylation of body proteins such as hemoglobin and albumin is the main cause of pathogenesis in chronic complications of diabetes. Protein glycosylation is an oxidative reaction. Antioxidants such as vitamin C may be able to reduce the chronic complications of diabetes through inhibiting protein glycosylation. The inhibitory effects of vitamin C and the polyphenolic extracts of *Betula pendula*, *Salvia hydrangea* and *Crataegus curvisepala* on the extent of glycosylation of albumin, insulin and hemoglobin were investigated in this study.

METHODS: Polyphenolic extracts of the aforesaid plants were prepared at three different concentrations, namely 3.6, 1.8 and 0.9 mg/ml. Vitamin C solutions were also prepared at five concentrations, namely 0.5, 5, 10, 50 and 500 µg/ml.

RESULTS: The highest degree of glycosylation inhibition of albumin and insulin was due to S. hydrangea, by 100% and 97% respectively, and that of hemoglobin was due to B. pendula by 80%. At its highest concentration, vitamin C inhibited the glycosylation of insulin, albumin and hemoglobin by 100%, 93%, and 58% respectively ($P<0.05$).

DISCUSSION: Based on our findings, the studied plants might be able to prevent the chronic complications of diabetes.

Keywords • Polyphenolic extract • Glycosylation • Antioxidants • Albumin • Insulin • Hemoglobin • Vitamin C • Crataegus • Salvia • Betula

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Introduction

Diabetes is one of the most common endocrine diseases spreading rapidly in the world. The condition is associated with increases in plasma glucose level, thickening of capillary basement membrane, and disturbances in protein and lipid metabolism. Non-inflammatory retinal diseases, nephropathy and accelerated atherosclerosis are among the clinical presentations of the disease, which are mainly due to non-enzymatic glycosylation of lipoproteins.[1-3] Thus, inhibition of this reaction is essential to decreasing the chronic complications of diabetes. Previous studies have demonstrated that flavonoids have remarkable inhibiting effects on protein glycosylation.[4,5]

In this study, the effects of three flavonoid-rich plants, namely *Betula pendula*, *Salvia hydrangea* and *Crataegus curvisepala* on protein glycosylation were assessed. *Betula pendula* contains quercetin, hyperoside, arabinopyranoside, and quercitrin.[6-9]

Crataegus curvisepala not only has antioxidant effects, but also contains flavonoids such as rutin, hyperoside, vitexin, and isoquercitrin.[10-12] It has recently been used as an antihypertensive drug in the treatment of cardiovascular disease.[13]

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Salvia hydrangea contains different flavonoids, strong antioxidants which are used in the treatment of diabetes. Given the inhibitory effects of pure flavonoids on protein glycosylation, this study was designed to determine the effect of polyphenolic extracts of the above-mentioned plants, as well as vitamin C as an antioxidant on the extent of glycosylation of albumin, hemoglobin and insulin.

Materials and methods

1. Preparation of protein solutions

a) *Hemoglobin*: Blood samples of healthy volunteers were collected using EDTA as an anticoagulant; hemoglobin was separated, and hematocrit value was determined using a cell counter.[14, 15]

b) *Albumin*: Human albumin (sigma 5g) was diluted with 0.1 Molar phosphate buffer (pH=7.4) to prepare a 0.05% solution.

c) *Insulin*: Regular bovine insulin (100 Iu/ml) was obtained from Lilly Company.

d) *Vitamin C*: 500 mg/5ml vials of vitamin C were obtained from Osvah Iran Company.

2. Preparation and extraction of polyphenolic extract

Aerial parts of *C. curvisepala* were collected from Hamgin region, Isfahan. Leaves of *Betula pendula* were obtained from Phillips University in Marburg (Germany) and the aerial parts of *S. hydrangea* were collected from Dehaghan region. All plants were identified and scientifically confirmed. A polyphenolic extract of each plant was prepared using 50 g dried powder and ethanol.[8] Stock solution was prepared from the extracts and the total flavonoid content was determined quantitatively via the spectrophotometric method.[16] Each test was repeated five times.

3. Glycosylation tests

The extent of glycosylation of each protein (hemoglobin, albumin and insulin) was determined in the presence or absence of different concentrations of each plant extract or vitamin C.[17]

Solutions containing polyphenolic extracts of each plant were prepared at three different concentrations, namely 3.6, 1.8, and 0.9mg/ml. Triplicates were used in all cases. The inhibitory effect of vitamin C (0.5, 5, 10, 50 and 500 µg/ml) was determined and compared with the control group.

4. Statistical analysis

The results are presented as means ± standard deviation (SD). The differences between the test and control groups were determined using Student's t-test ($P<0.05$).

Results

The optimum incubation time (72 hours) and the optimum glucose concentration (300ml/100mg) were known from previous studies.[4] The percentage glycosylation inhibition of proteins (hemoglobin, albumin and insulin) by polyphenolic extracts of each plant and vitamin C are shown in Tables 1 and 2.

Comparison of the inhibitory effect of plants on protein glycosylation showed the effect to be dose-dependent. The highest glycosylation inhibitory effects for albumin, hemoglobin and insulin were achieved by *S. hydrangea* (100 %), *B. pendula* and vitamin C (100%), respectively.

TABLE 1. Effect of plant extracts on the extent of albumin, hemoglobin and insulin glycosylation

Plant powder mg/ml		Flavonoid concentration (µg/ml)	Glycosylation inhibition (%)		
			Hemoglobin	Albumin	Insulin
B. pendula	(3.6)	1368.0	80	69	87
	(1.8)	684.6	54	58	46
	(0.9)	342.6	45	52	14
S. hydrangea	(3.6)	210.8	51	100	97
	(1.8)	105.4	33	97	65
	(0.9)	52.7	13	93	14
C. curvisepala	(3.6)	464	65	99	85
	(1.8)	232	43	97	43
	(0.9)	116	15	88	35

One milliliter of a 3000mg/1000 ml glucose solution containing 20 mg/100 ml gentamycin in a 0.01 M phosphate buffer (pH=7.4) was incubated with 1 ml of each protein solution and 0.1 ml of each plant extract for 72 hours. The extent of glycosylation inhibition was determined and compared with the control group.

TABLE 2. Effect of vitamin C on the extent of albumin, insulin and hemoglobin glycosylation

Vitamin C solution (μg/ml)	Absorbance at wavelength of 443 nm	Glycosylation inhibition %		
		Hemoglobin	Albumin	Insulin
500	0.206±0.0002	58	93	100
50	0.320±0.0001	63	87	100
10	0.358±0.0001	73	74	82
5	0.381±0.0003	23	76	74
0.5	0.378±0.0003	23	51	56

One milliliter of a 3000 mg/1000 ml glucose solution containing 20 mg/100ml gentamycin in a 0.01 M phosphate buffer (pH=7.4) was incubated with 1 ml of each protein solution and 0.1 ml of vitamin C for 72 hours. The extent of glycosylation inhibition was determined and compared with the control group.

Since the effect of these plants was dose-dependent, only the highest doses were used for comparisons.

The results are as follows:

A) Glycosylation of albumin was inhibited by *S. hydrangea*, *B. pendula*, *C. curvisepala* and vitamin C, by 100%, 69%, 99% and 93% respectively.

B) Glycosylation of hemoglobin was inhibited by *B. pendula*, *C. curvisepala*, *S. hydrangea* and vitamin C by 80%, 58%, 51% and 58% respectively.

C) Glycosylation of insulin was inhibited by *S. hydrangea*, *B. pendula*, *C. curvisepala* and vitamin C by 97%, 87%, 81% and 100% respectively.

Discussion

Diabetes mellitus is one of the most common endocrine diseases. It is estimated to be the fifth cause of death in the US. Most diabetes complications result from increased plasma glucose levels due to non-enzymatic glycosylation of body proteins.[18-21]

In previous studies, the antioxidant effects of pure flavonoids on glycosylation of hemoglobin, albumin, and insulin have been studied.[4] In this study, the effects of three plant species, namely *B. pendula*, *C. curvisepala* and *S. hydrangea*, which are important sources of flavonoids, were assessed.

The results show that the polyphenolic extracts of the plants studied at concentrations lower than that of vitamin C inhibit glycosylation to an extent comparable to this vitamin (especially in the cases of *S. hydrangea* and *C. curvisepala*).

Not only the quantity of polyphenolic compounds, but also their structure is important in inhibiting protein glycosylation. For example, *B. pendula* exhibited lower inhibitory effects on insulin glycosylation despite containing more flavonoids than other plants. Vitamin C is known as a strong antioxidant.[22] This vitamin is available only in a water soluble form,[23] while polyphenolic extracts of plants with hydrophobic and lipophilic properties have anti-oxidant properties comparable to those of vitamin C; hence they are of special significance as anti-oxidants.

As there are quantitative and qualitative differences between the flavonoids, one can compare their antioxidant effects by studying the relationship between their chemical structure and function.

Structural differences exist between albumin, hemoglobin and insulin molecules; hence their active moieties may react differently with antioxidants. The observed differences can thus be explained.

Quantitative and qualitative studies of the polyphenolic extracts of the three plants are warranted. The relationship between the molecular structure of proteins and the inhibitory effects of flavonoids on glycosylation should also be studied more extensively.

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