

Effect of Some High Consumption Spices on Hemoglobin Glycation

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Naderi, *et al.*: Antiglycation Properties of Spices

Formation of glycation products is major factor responsible in complications of diabetes. Worldwide trend is toward the use of natural additives in reducing the complications of diseases. Therefore, there is a growing interest in natural antiglycation found in plants. Herbs and spices are one of the most important targets to search for natural antiglycation from the point of view of safety. This study investigated the ability of some of the spices to inhibit glycation process in a hemoglobin/glucose model system and compared their potency with each other. For this subject the best concentration and time to incubate glucose with hemoglobin was investigated. Then the glycosylation degree of hemoglobin in the presence of extracts by the three concentrations 0.25, 0.5 and 1 µg/ml was measured colorimetrically at 520 nm. Results represent that some of extracts such as wild caraway, turmeric, cardamom and black pepper have inhibitory effects on hemoglobin glycation. But some of the extracts such as anise and saffron have not only inhibitory effects but also aggravated this event and have proglycation properties. In accordance with the results obtained we can conclude that wild caraway, turmeric, cardamom and black pepper especially wild caraway extracts are potent antiglycation agents, which can be of great value in the preventive glycation-associated complications in diabetes.

Key words: Glycosylation, hemoglobin, wild caraway, turmeric diabetes

Recently, there is strong evidence to show that diabetes is associated with increased oxidative stress and diabetic patients are more susceptible to oxidative attack than normal subjects^[1,2]. Oxidative stress occurred during hyperglycemia produces glycated proteins especially glycated hemoglobin and advanced glycated-end products. The accumulation of these products in living organisms leads to structural and functional modifications of proteins^[3].

Animal studies show that glycation inhibitors such as aminoguanidine attenuate vascular complications of diabetic patients^[4]. Therefore, using antioxidant supplements to suppress the glycation pathways may be beneficial for preventing diabetic complications. Results of some studies indicated that intake of antioxidants can reduce development of type 2 diabetes which support the hypothesis^[5,6].

Spices have been used as flavoring, coloring and preservatives agents for thousands of years. Also spices have shown medicinal properties and influence

on various body systems such as gastrointestinal, cardiovascular and reproductive and nervous systems^[7]. Researchers found antioxidants effects for some spices like *Pimpinella anisum*, *curcuma longa*, *Crocus sativus l* and *Piper nigrum*, *Ellitaria cardamom*^[8-11], but antiglycation properties of these spices haven't demonstrated.

So, as oxidation occurred during protein glycation, it seems that the spices with antioxidant properties have antiglycation effects. Therefore, we examined the inhibitory effects of hydroalcoholic extract of some spices on glycation and oxidation-dependent damages to hemoglobin induced by glucose.

Anise, cinnamon, clove, wild caraway, tanners sumach, turmeric, saffron, cardamom, black pepper were purchased at a local market in Isfahan City, Iran. These spices were authenticated at faculty of Pharmacy, Isfahan University of Medical Science, Isfahan, Iran. The voucher specimen of them was deposited in this place. After grounding with electronic blender, they were soaked in methanol 70° for 72 h. Then extracts were filtered with Whatman no. 1 filter paper and dried. The dried extracts were stored at 4° until used^[12].

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The red blood cells washed thrice with 0.14 M NaCl solution and were lysed with CCl_4 in phosphate buffer (0.01 M, pH=7.4). After lysing, the hemolysate was freed from debris by centrifugation. The supernatant was separated and its hemoglobin concentration was measured by the Drabkin method.

To find the best concentration of glucose, 5 g hemoglobin/100 ml phosphate buffer (0.01 M, pH 7.4) was incubated in the presence of different concentrations of glucose and the amount of glycosylation was measured by colorimetric method. Then, the hemoglobin was incubated with the best concentration of glucose at different times and the best time for glycosylation was determined.

After that, hemoglobin was incubated with the best concentration of glucose (10 mg/ml) and gentamycin (100 $\mu\text{g}/\text{ml}$) in the presence of different concentration of extracts (0, 0.25, 0.5 and 1 $\mu\text{g}/\text{ml}$) for 72 h (the best time for glycosylation). Then, the protein was precipitated with 20% trichloroacetic acid and treated with 0.5 ml oxalic acid in 1 ml phosphate buffer (0.01 M, pH 7.4) for 1 h at 100° . After cooling, trichloroacetic acid 40% was added and the solution centrifuged. Free sugars and hydroxymethyl furfural in the supernatant were treated with thiobarbituric acid for 30 min at 40° and a colored product with a maximum absorbance at 443 nm was yield. Comparing the colored product absorbance with control sample (a sample without extract), glycation inhibition percent of each extract was calculated

according to the formula Percent inhibition of glycation= $100 \times (A_A - A_B) / A_A$, where A_A and A_B are the absorbance values of the blank and of the tested samples^[13].

Data were analysed using SPSS software (version 15.0). Glycation inhibition percent values were expressed as means \pm standard deviations of triplicate determinations. One-way Analysis of Variance (ANOVA) and Tukey as Post Hoc test were used to do comparisons between different extract. Student T test were used to do Comparisons between each extract and control. Probability values of less than 0.05 were considered to be significant.

Quantitative results of the isolated extracts from the analysed spices have been summarized in (Table 1). In each 100 g of the dried spices, dried extracts weights vary from 4.92 g (cinnamon) to 59.55 g (saffron). Inhibitory effects of extracts on glucose-mediated glycation have been presented in (Table 1). Despite of anise and saffron the extract of wild caraway, turmeric, cardamom and black pepper were effectively able to reduce the amount of end product of hemoglobin glycosylation. While for cinnamon, clove and sumach this effect was average.

Inhibition effects of different concentrations of each extract on hemoglobin glycation have been compared separately (fig. 1), and except for black pepper and cardamom, significant difference was observed for other extracts.

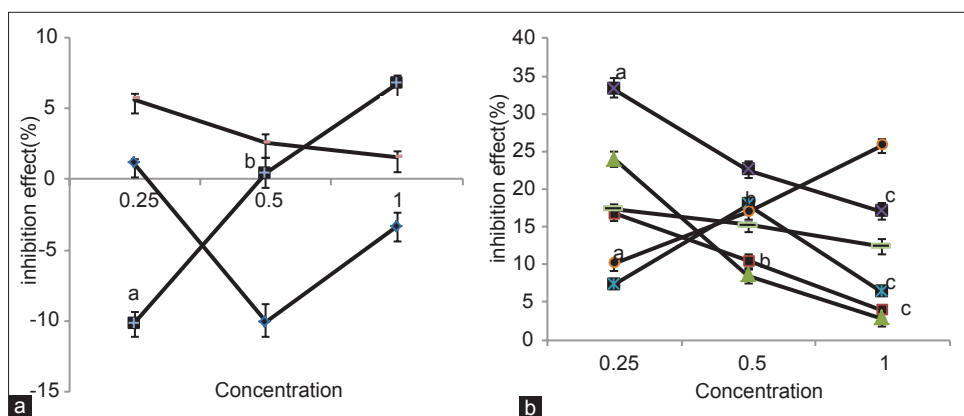


Fig. 1: Comparison between inhibition effects on concentration variable.

(A) Spices with or low effect or without effects on hemoglobin glycation, anise (◆), saffron (■) and cardamom (—). (B) Spices with required inhibition effects on hemoglobin glycation, cinnamon (■), clove (σ), wild caraway (o), tanner sumach (π), turmeric (□) and black pepper (—). Similar characters: insignificant differences, no similar characters: significant differences.

TABLE 1: INHIBITORY EFFECTS OF EXTRACTS ON HEMOGLOBIN GLYCATION

Plant	Common name	family	Part used	Extract yield (%)	Concentration ($\mu\text{g/ml}$)	Inhibition effect (%)	P value*
<i>Pimpinella anisum</i>	Anise	Umbelliferae	Branch	14.06	0.25	1.17 \pm 0.3	-
					0.5	-9.97 \pm 1.3	-
					1	-3.25 \pm 0.9	-
<i>Cinnamomum zeylanicum</i>	Cinnamon	Lauraceae	Derm	4.92	0.25	16.35 \pm 0.9	Sig
					0.5	10.54 \pm 0.8	-
					1	3.73 \pm 0.3	-
<i>Syzygium oromaticum</i>	Clove	Myrtaceae	Bud	19.18	0.25	23.82 \pm 1.0	Sig
					0.5	8.52 \pm 0.7	-
					1	2.74 \pm 0.5	-
<i>Bunium persicum</i>	Wild caraway	Umbelliferae	Fruit	11.01	0.25	33.12 \pm 1.6	Sig
					0.5	22.60 \pm 1.1	Sig
					1	17.21 \pm 1.2	Sig
<i>Rhus coriaria</i>	Tanners sumach	Anacardiaceae	Fruit	30.95	0.25	7.48 \pm 0.7	Sig
					0.5	17.76 \pm 1.1	Sig
					1	6.6 \pm 0.5	-
<i>Curcoma longa</i>	Turmeric	Zingiberaceae	Rhizomes	10.91	0.25	10.22 \pm 0.4	-
					0.5	16.99 \pm 0.7	Sig
					1	25.83 \pm 0.7	Sig
<i>Crocus sativus</i>	Saffron	Iridaceae	stigma	59.55	0.25	-10.15 \pm 0.4	-
					0.5	0.42 \pm 0.1	-
					1	6/74 \pm 0.8	-
<i>Elettaria cardamomum</i>	Cardamon	Zingiberaceae	seed	5.60	0.25	5.73 \pm 0.4	Sig
					0.5	2.6 \pm 0.6	-
					1	1.6 \pm 0.5	-
<i>Piper nigrum</i>	Black pepper	Piperaceae	seed	9.78	0.25	17.35 \pm 0.5	Sig
					0.5	15.17 \pm 0.2	Sig
					1	12.38 \pm 0.9	Sig

*Comparison between inhibition effects on hemoglobin glycation of each extract as compared control (without extract). Sig is significant, - is not significant

According to the (fig. 2), in concentration 0.25 $\mu\text{g/ml}$ wild caraway with 33.12% and anise with 1.17% showed the highest and the lowest inhibition effects, respectively. Saffron in this concentration had proglycation effect (-10.15%). The highest inhibition percent was observed in concentration of 0.5 $\mu\text{g/ml}$ of wild caraway, turmeric and tanners sumach (33.19, 16.99 and 17.76%) and the same concentration of anise showed proglycation effect (-9.97 \pm 1.30). Turmeric (1 $\mu\text{g/ml}$) with 25.83% and Anise with -3.25% had the highest and the lowest inhibition effects on hemoglobin glycation, respectively.

According to our results some of the extracts have significantly inhibitory effects on hemoglobin glycation. The percent of inhibition vary from 33.12% (wild caraway in 0.25 $\mu\text{g/ml}$) to 0.42% (saffron 0.5 $\mu\text{g/ml}$). Some of the extracts have proglycation properties as 0.25 $\mu\text{g/ml}$ of Saffron (-10.15%) and anise at 0.5 $\mu\text{g/ml}$ (-9.97%).

These different effects may be originated from the existence of various compounds in these extracts that have different effects.

Glycosylation of haemoglobin takes place under physiological condition by a reaction between glucose and N-terminal valine of haemoglobin molecule's beta chain^[14]. The oxidative steps are also involved in glycation and the process can therefore be called glycooxidation^[15]. Regarding the presence of free radicals and oxidative steps in the glycooxidation process, At first glance, it seems that the antiglycation activities of the extracts are due to the antioxidant properties^[16].

In research studies, the presence of antioxidant compounds were proved in all these spices, as coumarin, cinnamaldehyde in cinnamon, curcumin in turmeric, eugenol in cardamom and sesquiterpenoid in black pepper. But some of these spices can't inhibit hemoglobin glycation as anise^[17].

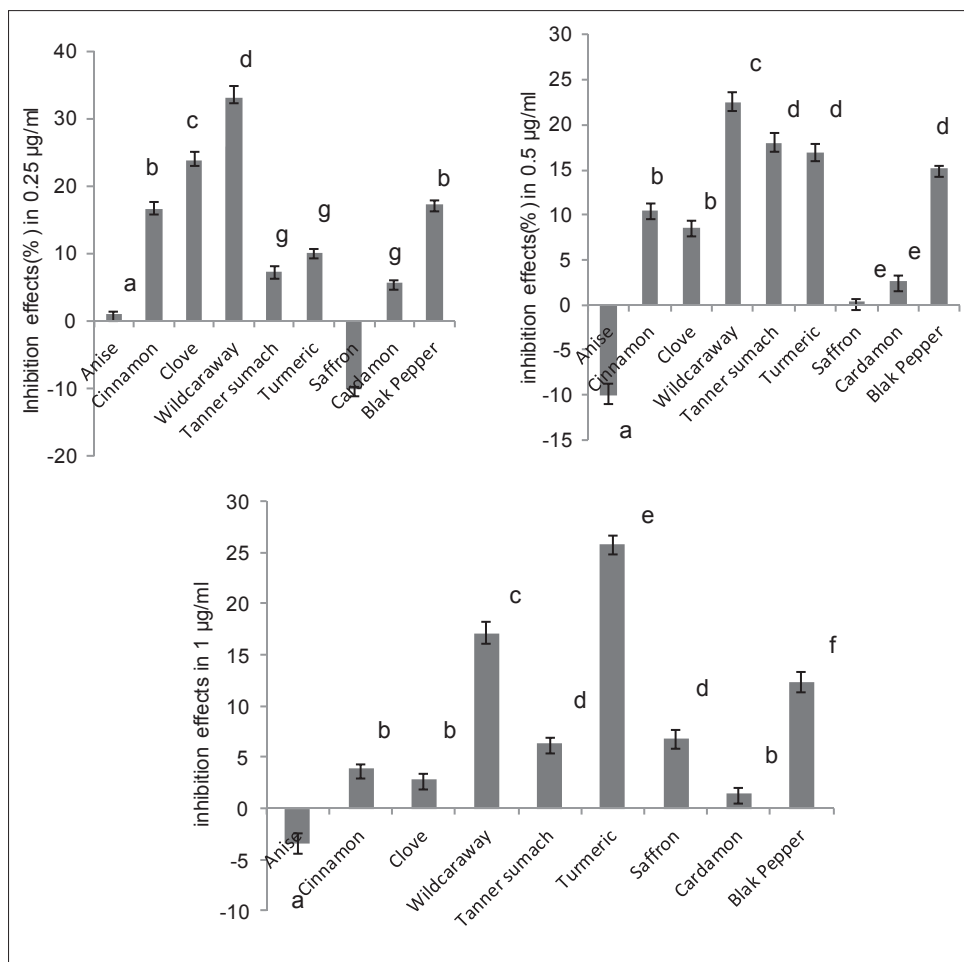


Fig. 2: Comparison between inhibition effects on extract variable.
Similar characters: insignificant differences, Different characters: significant differences.

In this study Anise couldn't effectively reduce hemoglobin glycation however its antioxidant activity has been obtained. So that, this spice effectively scavenged nitric oxide, superoxide and DPPH radicals and it also decrease lipid peroxidation and protein oxidation in diabetics^[8]. In β -carotene bleaching system antioxidant activity of the essential oil of anise was almost equal to butylated hydroxytoluene (BHT)^[16]. These antonyms can be having different reasons. For example, the flavonoids as the most important antioxidant components, have invert-U effects and act with different intensity^[19]. This means that the maximum activity of each flavonoid is at a particular dose and at higher and lower doses than this dose, the activity is descended.

Moreover, other mechanisms beside the scavenging free radicals may be responsible for this inhibition like metal-chelating property. Moreover, the metal-chelating property of extracts has been proven

to be responsible for alleviation of the Amadori reaction in glycation system. The rationale behind this speculation includes the previous report that, diethylenetriaminepentaacetic acid, a known metal-chelating activity, reduced albumin glycation^[20]. Also, a strong relationship between glucose associated oxidative modifications and amount of transition metals like copper and iron was seen^[21]. So, the desirable effects of some of the extracts like wild caraway may be related to the other mechanisms apart from scavenging free radicals.

In conclusion we can say that in addition of antioxidant activity, there are different mechanisms for inhibition of glycation and likely a mixture of these mechanisms participate in this effect.

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Accepted 26 September 2014
Revised 22 September 2014
Received 11 September 2013
Indian J Pharm Sci 2014;76(6):553-557