STUDY ON THE EFFECT OF GARLIC ON THE IN VITRO ALBUMIN GLYCATION REACTION

N. Sheikh[1], M.R. Safari[1], Kh. Mani Kashani[2], M. Araghchian[3] and F. Zeraati[3]

1) Department of Biochemistry and Nutrition, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

2) Department of Social Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

3) Department of Pharmacology, School of Medicine, Hamadan University of Medical Science, Hamadan, Iran

Abstract- Garlic, an antioxidant plant, can react with amino groups of proteins to form Schiff bases. As diabetes leads to glycation of various proteins and this in turn has some effects on the structure of proteins and biochemical activity of them, the inhibition of this process seems very vital. For several years researchers in this field have done their best to recognize the antidiabetic compounds. The aim of this study is to determine the effects of garlic on albumin glycation *in vitro*.In the presence of various concentrations of garlic, albumin was glycated and evaluated using TBA (thio-barbituric acid) method. The results showed that garlic has a statistically significant (P<0.05) effect in inhibiting or decreasing the reaction of albumin glycation. The findings of this research shows that garlic probably inhibits the reaction of glycation and decreases complications occurring in diabetes.

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Key words: Albumin glycation, garlic, *in vitro*

INTRODUCTION

Garlic is a common food additives and spice used in Chinese, French, Thai, Cajon, Italian and many other cultures. Garlic is a minor source of selenium, chromium, potassium, germanium, calcium, iron and vitamin A, C and B complex (1). The aroma, flavor and medical properties of garlic are primarily the result of sulfur compounds including aliin, ajoen and alicin (2). Garlic inhibits lipid synthesis, thus decreasing cholesterol and triglyceride levels. It has been considered for a long time that hyperglycemia is primarily the leading pathogenetic factor of micro-angiopathy and it has been supposed that microangiopathy is the specific complication of diabetes mellitus. Chronic hyperglycemic state of the organism predetermines the intensification of fermentative and non-fermentative glycation (joining of glucose to the amine NH2 group of lysine residue) of blood proteins and lipids. End products of glycation irreversibly connected with proteins can significantly change their properties. As a result the change of function and endothelial vascular wall damage takes place (3-5).

Studies have shown that garlic extract may prevent diabetic cardiovascular complications and it is reported that daily oral feeding of garlic extract could increase the cardiovascular function in diabetic rats. Also, garlic extract could increase fibrinolitic activities with the decrease of platelet aggregation (6). Also, it is proved that garlic lowers the blood glucose level by increasing the body's circulating insulin and by increasing glycogen storage in the liver (2). In diabetic patients, some of the chronic complications occurring are thought to be due mainly to the subsequent reactions of glycated proteins and using the inhibitors could have positive effects in reducing chronic complication occurring in diabetes (7-10). In this research the effect of different concentrations of garlic on *in vitro* albumin glycation has been studied.

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*** Corresponding Author:**
N. Sheikh. Department of Biochemistry and Nutrition, Faculty of Medicine. Hamadan University of Medical Sciences, Hamadan, Iran
Tel: +98 811 8256295, Fax: +98 811 8276299
E-mail: sheikh@umsha.ac.ir

MATERIALS AND METHODS

Materials

Garlic powder has been obtained from Golpar-Sina Co. and all other chemical materials have been

prepared from Merck company (Germany) and Sigma company (USA).

Test method

1. The albumin glycation reaction:

One ml of 3000 mg/100ml glucose solution was added to 1 ml of 5 g/dl albumin solution. For the prevention of any environmental contamination Gentamicin with the concentration of 20 mg/100 ml was added to 0.01 mol/L phosphate buffer (pH=7.4) and incubated 72 hours in room temperature in constant position. Then, after the incubation period, it was dialyzed in phosphate buffer (the dialyzed bag was prepared in 10 mmol/L EDTA before this process) (11).

2. Measuring the level of albumin glycation :

For approving the albumin glycation TBA test was used, as follows: One ml 20% trichloro acetic acid (TCA) was added to above solution and then centrifugated for 10 minutes at 3000 rpm. The supernatant was discharged. This function was done twice. 1 ml phosphate buffer with above specification and 0.5 ml 0.3N oxalic acid were added to the sediment and put in boiling conditions in water bath. After the compound got cold in lab temperature 0.5 ml 40% TCA was added to each sample. After centrifugating for 10 min at 3000 rpm, the supernatant was separated and 0.5 ml 5% M TBA was added to 1 ml of supernatant solution, then the whole was set in 40 degree water bath for half an hour. At the end, the absorbance of the sample was measured in 443 nm (12).

3. Preparing the stock solution of Garlic:

0.1 gr garlic was dissolved in 10 ml bidistilled water and used as garlic stock solution.

4. Determining the effect of garlic on albumin glycation:

Three different concentrations, 1gr/dl, 0.2 gr/dl and 0.1 gr/dl from garlic stock solution were prepared and 0.1 ml from different concentrations of garlic was added to a solution having 5% albumin and 3000 mg/100ml glucose (in Gentamicin phosphate buffer solution) and incubated in lab temperature for 72 hours. To determine the effect level of each concentration of garlic, TBA reaction was done as in method number 2. All of the experiment stages and each concentrations of garlic were done as triplet and for getting acceptable result the tests were repeated.

Statistical analysis

Data were expressed as mean ± standard deviation. One way analysis of variance was used to compare the effect of garlic on inhibiting albumin glycation reaction in terms of different concentrations and Tukey HSD test was used to assess differences in comparing pairs.

RESULTS

In this research the effects of the various concentrations of garlic on albumin glycation were studied. Various concentrations of garlic (0, 0.1, 0.2 and 1 gr/dl) were used in the test and by using TBA method the level of albumin glycation was determined. The results showed that garlic had potential inhibitory effect on albumin glycation. The following concentrations of 0.1, 0.2 and 1 gr/dl had the inhibitory effects of 56.1%, 68.1% and 79.1%, respectively (Table 1). The interaction of the different concentrations of garlic on inhibiting albumin glycation reaction showed that comparing concentrations of garlic in pairs would reveal each pair (1 g/dl-0.2 g/dl, 0.1 g/dl-1g/dl) had statistically significant difference (Table 2).

Table 1. Comparing the effect of garlic on inhibiting albumin glycation reaction in terms of different concentrations

garlic concentration	Level of inhibiting of albumin glycation (mean±SD)	Standard error of mean	F value	*P* value*
1 gr/dl	79.06 ± 2.28	1.28	F= 27.85 (6,2)	*P*= 0.001
0.2 gr/dl	68.07 ±1.75	1.01		Significant
0.1 gr/dl	56.14 ± 5.9	3.4		

Abbreviation: SD, Standard Deviation

***** One way analysis of variance

Table 2. Comparison of the different concentrations of garlic in pairs on inhibiting albumin glycation reaction in terms of different concentrations*

Comparing concentration (1)	Comparing concentration(2)	difference of mean	Standard error of mean	*P* value
Garlic 1 gr/dl	Garlic 0.2 gr/dl	10.991	3.08	0.028†
Garlic 1 gr/dl	Garlic 0.1 gr/dl	22.92	3.08	0.001†
Garlic 0.2 gr/dl	Garlic 0.1 gr/dl	11.93	3.08	0.019†

* Tukey HSD test

† Significant

DISCUSSION

This study confirmed that garlic affects the glycation of proteins. Garlic is a constituent with at least 23 sulfur identified compounds. Garlic is approved in Europe for decreasing atherosolerosis, cancer prevention, reducing peripheral arterial vascular disease and antibacterial and viral properties (1). Also, it decreases the blood glucose level. The studies of Sheela et al (13,14) showed that the oral administration of garlic (*Allium Sativum Linn*) by diabetic rats for a month decreased the complication of diabetes. Also, Augusti et al (9) reported that the antioxidant S-allyl cysteine sulfoxide isolated from garlic (*Allium Sativum Linn*) had significant anti diabetic effects. Roman- Ramos et al (10) studied the effects of 12 edible plants on diabetic patients and found that garlic (*Allium Sativum Linn*) decreased the hyperglycemic peak. Some of the previous observations have shown that garlic had the anti diabetic properties, but the effect of garlic on protein glycation is not obvious. In this study, we investigated the effects of garlic on the albumin glycation, and among the various concentrations, 1gr/dl had the highest inhibitory effect. In conclusion, these effects highlight the therapeutic value of garlic.

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