# Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

*JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.*

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J. Med. Sci., 7 (6): 1039-1043
15th August, 2007

# The Inhibitory Effect of Garlic Extract on Formation of Glycated Hemoglobin and AGEPs

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In this study human hemoglobin in phosphate buffered saline (pH 7.4) was incubated with 20 mmol $L^{-1}$ glucose, in order to identify the Antioxidant Effect of Garlic (AGE) on formation of Advanced Glycation End Products (AGEPs). Different concentration of garlic extract (0.1, 0.3, 0.5 and 1 g $dL^{-1}$) at 1 to 60 days of incubation were investigated. AGEPS was quantified by autofluorecence characterization and Hb-A1C was separated from the hemoglobin by cation exchange chromatography. The increase in glycated hemoglobin levels was blocked significantly when Hemoglobin was pretreated with garlic extract (0.5 g $dL^{-1}$). Present study indicated that Garlic extract inhibit the formation of AGEPs. These results suggested that the inhibitory effect of garlic extract on formation of glycated hemoglobin and AGEPs could be attributed to the antioxidant activity of garlic extract.

**Key words:** Glycation, garlic extract, Hb-A1C, AGEPs

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## INTRODUCTION

Garlic (*Allium sativum*) has been considered a beneficial therapeutic agent for many years (Kendler, 1987). Several studies in humans have demonstrated that garlic has beneficial effects on blood pressure (Auer *et al*., 1990), platelet aggregation (Brigges *et al*., 2000) and fibrinolytic activity (Bordia *et al*., 1977). Garlic modulates the inflammatory and controlles lipid peroxidation (Augusti and Sheela, 1996). In fact, Allium species are rich sources of phyto nutrient, which can useful for the treatment/prevention of some disease; including cancers, coronary heart diseases, obesity, hypercholesterolemia, diabetes types (Kennedy and Lyons, 1997; Rahman *et al*., 2006).

Diallyl sulfide, diallyldisulfide, s-ethyl cysteine and N-acetyl cysteine are four organo sulfur compounds derived from Allium plants such as garlic and onion. Recent studies have been suggested that these four organosulfur compounds might be contributed to the antioxidant activity of garlic extract (Yin *et al*., 2002).

The well-described non-enzymatic reaction of reducing sugars with protein side chains, lipids, or nucleic is named glycation. Products formed during long-term incubation in complex multistep reaction and rearrangements are termed Advanced Glycation End product (AGE) (Bucala *et al*., 1994). *In vivo*, AGEs were found to contribute to the onset of several diseases such as diabetic complication (Bucala *et al*., 1994; Kennedy and Lyons, 1997) renal insufficiency and Alzheimer disease (Kennedy and Lyons, 1997; Vitek *et al*., 1994). Glycation and Advanced Glycation End Products (AGEPs) formation are also accompanied by formation of free radicals via autoxidation of glucose and glycated proteins (Ahmad and Ahmed, 2006; Hunt *et al*., 1988).

In the present study, the role of inhibitory Effect of Garlic Extraction (AGE), on formation of Hb-A1C and AGEPs was investigated.

## MATERIALS AND METHODS

This study was conducted in Tehran University of Medical Sciences, Department of Biochemistry (2005-2006).

**Chemicals:** Hemoglobin obtained from a healthy volunteer was washed with saline. Garlic has been obtained from Hamedan Grandis Co (Iran). All other chemicals were obtained from the Sigma Chemical Co.

**Preparation of garlic extract:** Garlic (*Allium sativum* Linn) bulbs were cut into small pieces. About 250 mL of distilled water per 100 g of garlic were added and the mixture crushed in mixing machine. The resultant slurry was squeezed and filtered through a 0.22 μm filter. Then concentrated in a rotary vacuum evaporator (T<40°C) until all extraction solvent was completely removed.

***In vitro* incubation with glucose:** Human hemoglobin, D (+) glucose and 4 mol $L^{-1}$ sodium phosphate buffer (pH = 7.4) were used for the *in vitro* experiment. All solution was made in deionized water. Different concentration of glucose (5 and 20 mmol $L^{-1}$) was used for incubation of hemoglobin to have the normoglycemic and hyperglycemic conditions respectively, while the control solution lacked glucose. Hemoglobin at a concentration of 50 mg $mL^{-1}$ was dissolved in 0.4 mol $L^{-1}$ sodium phosphate buffer (pH = 7.4) and filter-sterilized by using a 0.22 μm Millipore filter. The samples were incubated in a rotary-shaking water bath at 37°C for 60 days under sterile and dark condition. The samples were withdrawn at weekly intervals and diluted to obtain the final concentration of 1.0 mg $mL^{-1}$ with 0.4 mol $L^{-1}$ sodium phosphate buffer (pH = 7.4) (Gopalkrishnapillai *et al*., 2003; Nadanathangam *et al*., 2005).

***In vitro* treatment with garlic extract:** In order to examine the protective role of Garlic extract against glycation of hemoglobin, hemoglobins were incubated with garlic extract of different concentration (0.1, 0.3, 0.5 g $dL^{-1}$) for 1 h at 37°C. Nonenzymatic glycation of hemoglobin was initiated by incubating the pretreated (with garlic extract) hemoglobin with glucose of 20 mmol $L^{-1}$. The contents were incubated in a shaking water bath at 37°C for 60 days.

**Measurement of glycated hemoglobin (Hb-A1C):** Glycated hemoglobin was measured by cation exchange chromatography (fast protein liquid chromatography system, MnoS HR 5/5 column at pH 5.7) and expressed as percentage of the total amount of hemoglobin (Nadanathangam *et al*., 2005).

**Analysis of AGEPs:** AGEPs fluorescence spectra were determined at $\lambda_{ex}$ = 308 nm, $\lambda_{em}$ = 345. Samples were diluted until 1.0 mg $mL^{-1}$ protein concentration (Gopalkrishnapillai *et al*., 2003).

**Statistical analysis:** All values were expressed as mean±standard error (SEM) of 3 samples. Analysis of variance (ANOVA) followed by student Newmans-Keuls test was used to evaluate the significancy of the results obtained. All computations were performed by computer using SPSS software.

## RESULTS

Different concentrations of garlic extract of 0.1, 0.3 and 0.5 g $dL^{-1}$ on formation Hb-A1C *In vitro* are examined. There is any significant difference between concentrations of 0.5 g $dL^{-1}$ with test group ($p<0.05$; Fig. 1). Different concentrations of garlic extract of 0.1, 0.3 and 0.5 g $dL^{-1}$ on formation AGEPs *in vitro* are examined. There is any significant difference between concentrations of 0.5 g $dL^{-1}$ with test group ($p<0.05$; Fig. 2).

Comparing positive test group with concentration of 0.5 g $dL^{-1}$ is seen that garlic at concentration of 0.5 g $dL^{-1}$

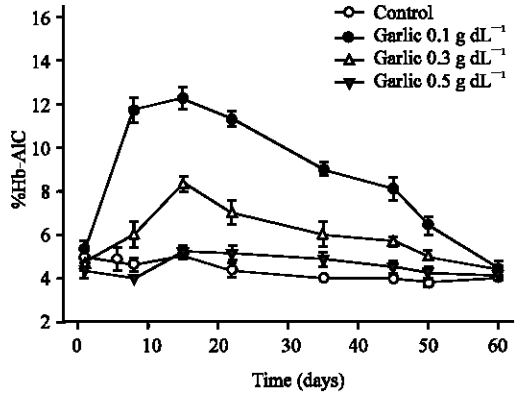


Fig. 1: Different concentrations of garlic extract of 0.1, 0.3 and 0.5 g $dL^{-1}$ on formation Hb-A1C *in vitro* are examined. There is any significant difference between concentrations of 0.5 g $dL^{-1}$ with test group. Data are the mean±SD of three experiments ($p<0.05$)

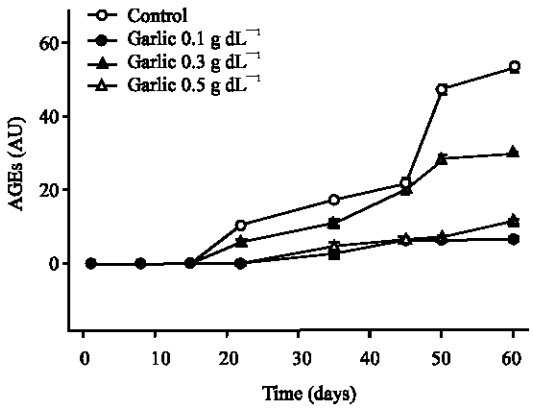


Fig. 2: Different concentrations of garlic extract of 0.1, 0.3 and 0.5 g $dL^{-1}$ on formation AGEPs *in vitro* are examined. There is any significant difference between concentrations of 0.5 g $dL^{-1}$ with test group. Data are the mean±SD of three experiments ($p<0.05$)

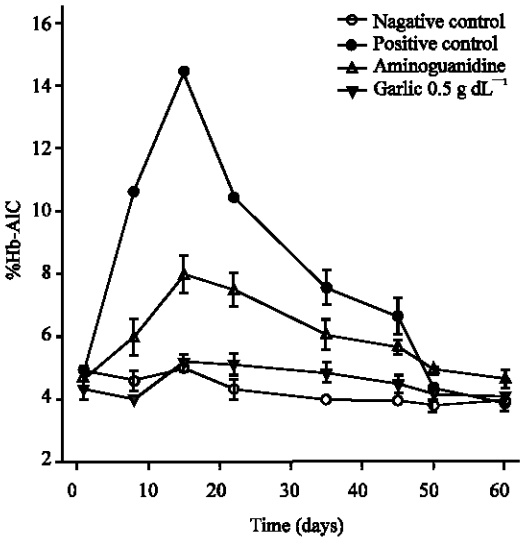


Fig. 3: Comparing positive test group with concentration of 0.5 g $dL^{-1}$ is seen that garlic at concentration of 0.5 g $dL^{-1}$ has a significant difference with positive test group in decreasing Hb-A1C up to time. Data are the mean±SD of three experiments ($p<0.05$)

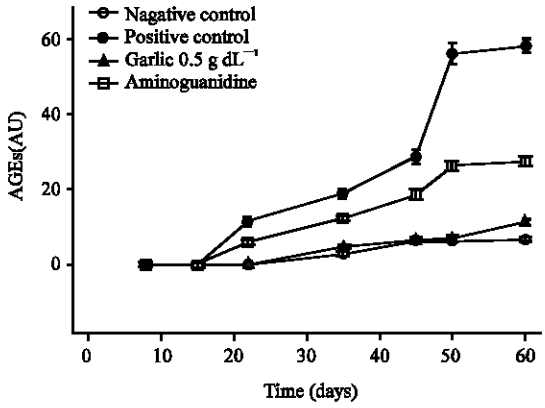


Fig. 4: Comparing positive test group with concentration of 0.5 g $dL^{-1}$ is seen that garlic at concentration of 0.5 g $dL^{-1}$ has a significant difference with positive test group in decreasing AGEPs up to time. Data are the mean±SD of three experiments ($p<0.05$)

have a significant difference with positive test group in decreasing Hb-A1C up to time ($p<0.05$; Fig. 3). Comparing positive test group with concentration of 0.5 g $dL^{-1}$ is seen that garlic at concentration of 0.5 g $dL^{-1}$ have a significant difference with positive test group in decreasing AGEPs up to time ($p<0.05$; Fig. 4).

## DISCUSSION

Many clinical and experimental observations have highlighted the important role played by the enhanced

nonenzymatic glycation of structural protein in contributing to the pathogenesis related to diabetes, CRF, atherosclerosis and aging. Spontaneous nonenzymatic modification of protein is commonly reported in tissues with slow turnover and they are considered by several authors as possible common mechanism involved in the progression of many pathological conditions (Vitek *et al*., 1994). Much effort has thus been spent searching for substances capable of either preventing or arresting the progression of glycation-dependent complications. A dietary antioxidant can be defied as substance in food that significantly decrease the adverse effects of reactive oxygen and nitrogen species, on normal physiological function in humans. Anti oxidants compounds such as vitamin C, vitamin E have been shown to have vascular-related effects that prevent or reverse nerve condition velocity deficits in experimental models. Garcinol from Garcinia have also shown to reduce *in vitro* protein glycation (Yamaguchi *et al*., 2000). There are reports of some of some natural substances isolated from plants with AGE-inhibitory effects. One such compound is S-allyl cysteine found in aged garlic. The major finding of the present study are prevention of glycation *in vitro* by garlic extract. Garlic extract inhibits *in vitro* oxidative modification of low-density lipoprotein by action scavenging oxidants (Hung, 2004; Yin, 2002).

We found that garlic extract (0.3 and 0.5 mg $dL^{-1}$) caused decrease in the levels of HbA1c in hemoglobins incubated with 20 mmol $L^{-1}$ glucose. Our results are consistent with the observations Sheikh *et al.* (2004) that supplementation of garlic in vitro can prevent glycation of albumin and LDL (Sheikh *et al*., 2004; Hung, 2004; Kim, 2003). Also pretreatment with garlic extract (0.3 and 0.5) significantly reduced the formation of AGEPs (Fig. 3). Were pretreated with aminoguandine the HbA1c was significantly attenuated similar to the effect of garlic extract, preincubation of hemoglobin with aminoguanidine significantly inhabited the AGEPs formation. In conclusion, this study has shown that, *in vitro*, garlic extract and aminoguanidine can inhibit the nonenzymatic glycation of hemoglobin and AGEPs.

## ACKNOWLEDGMENTS

This study was supported by a grant from Vice Chancellor of Research of Tehran University of Medical Sciences (March, 2005).

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