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A STUDY ON THE ROLE OF DIETARY AGENT AGED GARLIC EXTRACT ON PROTEIN GLYCATION

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ABSTRACT

Protein glycation is a spontaneous post translational modification of proteins by excess sugars causing formation of advanced glycation end products (AGEs) in diabetic individuals and responsible for diabetes complications. A wide variety of anti-glycating agents have been reported & recently there has been interest in natural products with anti-glycation properties. The Garlic has been used historically for medicinal purposes particularly for treatment of diseases associated with ageing. Aged garlic extract contains potent antioxidant activity and it can be a useful anti-glycating agent hence we planned for analyse the various concentrations of extract of aged garlic extract. Human serum albumin was used for *in vitro* glycation. The result demonstrated that Aged garlic extract has potent inhibitory effect on the protein glycation as revealed by dose dependent increase in fluorescence intensity in tryptophan fluorescence, decrease in AGE-

specific fluorescence, decrease in protein bound carbonyl groups, and a reduction in the intensity of the band in SDS-PAGE analysis. It concluded that Aged garlic extract nullifies fructose mediated cross-linking, carbonyl formation and AGEs formation as revealed by SDS-PAGE analysis, fluorescence spectroscopy and chemical estimation of carbonyl moiety in HSA.

KEYWORDS: AGEs- advanced glycation end products; Aged garlic extract; Serum albumin.

INTRODUCTION

Glycation is a spontaneous post-translational modification of proteins in which reducing sugars bind covalently to the free amino groups of proteins which leads to formation of advanced glycation end products (AGEs).[1-2] AGEs contribute to the onset of several diseases such as diabetic complications, renal insufficiency, and Alzheimer's disease.[3-4] Therefore, as another mode of prevention of diabetes complications will not be dependent on the control of blood glucose level. Thus far, some compounds such as aminoguanidine, aspirin, vitamin B6, taurine, quercetinand anti-inflammatory drugs including ibuprofen, are reported to be inhibitors of the glycation reaction.[5-10]

Garlic (*Allium sativum*) has been used historically for medicinal purposes, particularly for treatment of diseases associated with ageing.[11] Aged garlic extract contains potent antioxidant activity and is prepared from natural garlic that is aged for 20-months reducing its harsh irritating taste and odour. However, this aged garlic has a greater concentration of organo-sulphur compounds such as *S*-allyl cysteine which is a potent antioxidant and free radical scavenger.[12] Although numerous studies have demonstrated the antioxidant properties of aged garlic extract; its ability to inhibit formation of advanced glycation end products is unknown. Hence, our study endeavors to analyze the effect of aged garlic extract on *in vitro* HSA (Human Serum Albumin) glycation produced by fructose, a more potent AGE forming agent than glucose to induce more AGE formation in the system. Several studies have shown that the chemical inhibition of HSA glycation helps attenuate diabetic complications. Hence, the study can be useful in providing insight for prevention of secondary complications of diabetes.

MATERIALS AND METHODS

This study was undertaken in the Department of Biochemistry, J. N. Medical College, Aligarh Muslim University, Aligarh,Uttarpradesh,India.

MATERIALS

Human serum albumin (HSA), dinitro phenyl hydrazine (DNPH), ethylene tetradiamine tetra-acetic acid (EDTA), Coomassie brilliant Blue R-250, sodium dodecyl sulphate (SDS), agarose and dialysis membranes of one inch diameter were purchased from Sigma Chemical Company, U.S.A. Aged garlic extract was a product of Wakunaga Pharmaceutical Company, Tokyo, Japan.

EQUIPMENTS

Digital pH meter, type DPH-100, Shimadzu RF-5301 PC Spectrofluorometer, Beckman-DU-640B Spectrophotometer, Lyophilizer-HETO, Photochem-8 colorimeter, Microplate reader-Qualisystem PR-601, Polyacrylamide gel electrophoresis assembly (Genei Bangalore) were the major equipments used in this study.

Isolation of Plasma albumin

Plasma albumin was isolated by the method of Tayyab & Qasim (1990).[13] Human blood was procured from the Emergency operation theatre (O.T.)/Blood Bank of Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh.

Determination of protein concentration

Protein concentration was determined by the method of Lowry *et al,* (1951) using bovine serum albumin as the standard.[14] In this method of protein estimation, two reagents, namely, Folin & Ciocalteau's phenol reagent and copper reagent were used.

Gel chromatography

Sephacryl S-100 HR column was used for gel chromatography.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of HSA was carried out in tris-glycine buffer, pH 8.3 on 7.5 % polyacrylamide gels according to the method of Laemmli (1970).[15]

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was performed by the tris-glycine buffer system of Laemmli (1970) using slab gel electrophoresis apparatus.[15]

Staining Procedures

After the electrophoresis was complete the gels were removed and the protein bands were visualized by Coomassie brilliant blue staining.

Spectral Analysis

Ultraviolet absorption spectroscopy

The ultraviolet absorption spectra of native and glycated HSA samples were recorded in the wavelength range 200-400 nm on a Beckman-DU-640B spectrophotometer, using a cuvette of 1 cm pathlength. One mg of native and glycated HSA in a total volume of 3.0 ml was taken for spectral analysis.

Tryptophan Fluorescence

The fluorescence of tryptophan residue Trp214 in native and glycated HSA was monitored with excitation at 285 nm and the emission measured over the range 290-440 nm.[16] The concentration of protein samples was taken as 100 µM.

Advanced glycation end products (AGEs) related fluorescence

AGEs formations were measured by determining the fluorescence by excitation at 370 nm and emission between 400-500nm using Shimadzu RF-5301 PC spectrofluorophotometer.[17] The concentration of protein sample was taken as 100 µM.

Determination of protein bound carbonyl groups

HSA bound carbonyl groups were estimated by a published procedure.[18]

Determination of Total Phenolic content

Total phenolic content was determined by the method of Saucier *et al*., (1999)with slight modification and the results were expressed directly in absorbance units at 765 nm.[19]

RESULTS

Total polyphenolic compounds

The extract of Aged garlic was found to have good amount (60.3 mg/g) of polyphenolic compounds which is expressed in Gallic Acid Equivalent (GAE; mg/g).

Tryptophan Fluorescence

Glycated HSA showed decrease in fluorescent intensity as can be seen from Fig. 1, glycated HSA samples treated with Aged garlic extract showed increase in fluorescence intensity in a dose-dependent manner.

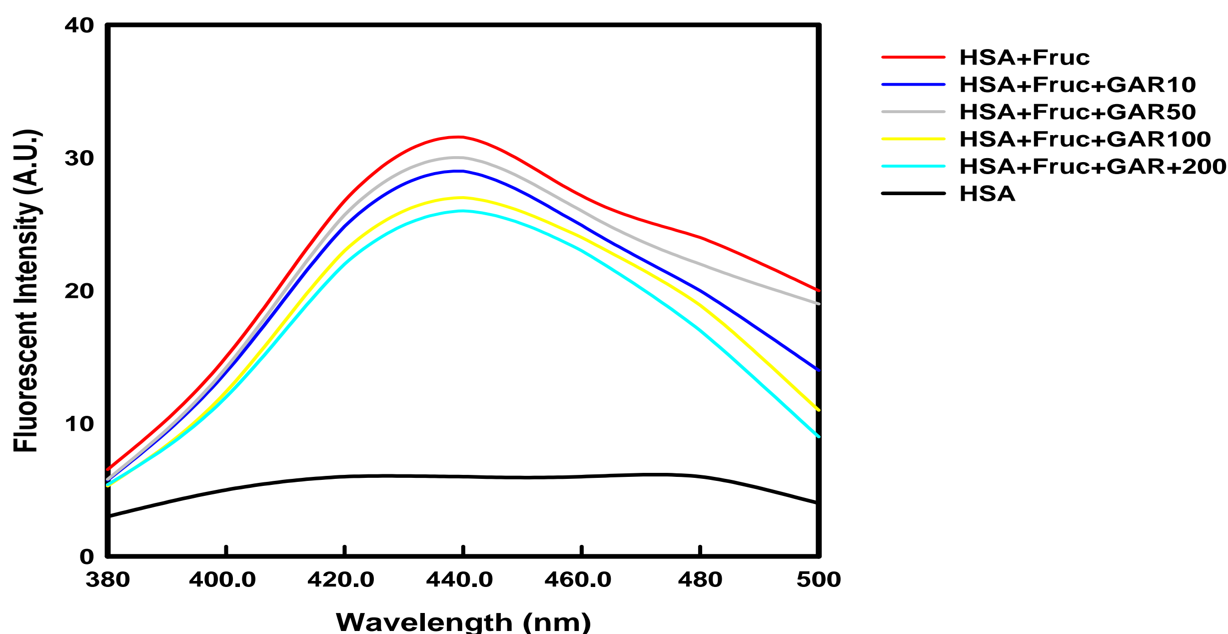

Fig. 1: Tryptophan specific fluorescence spectra of native, glycated and Aged garlic extract treated glycated HSA samples

AGE-Specific Fluorescence

AGE-specific fluorescence was found to increase in the presence of fructose following the 21-days incubation. Glycated HSA samples treated with Aged garlic extract showed a decrease in AGE-specific fluorescence in a dose-dependent manner (Fig. 2)

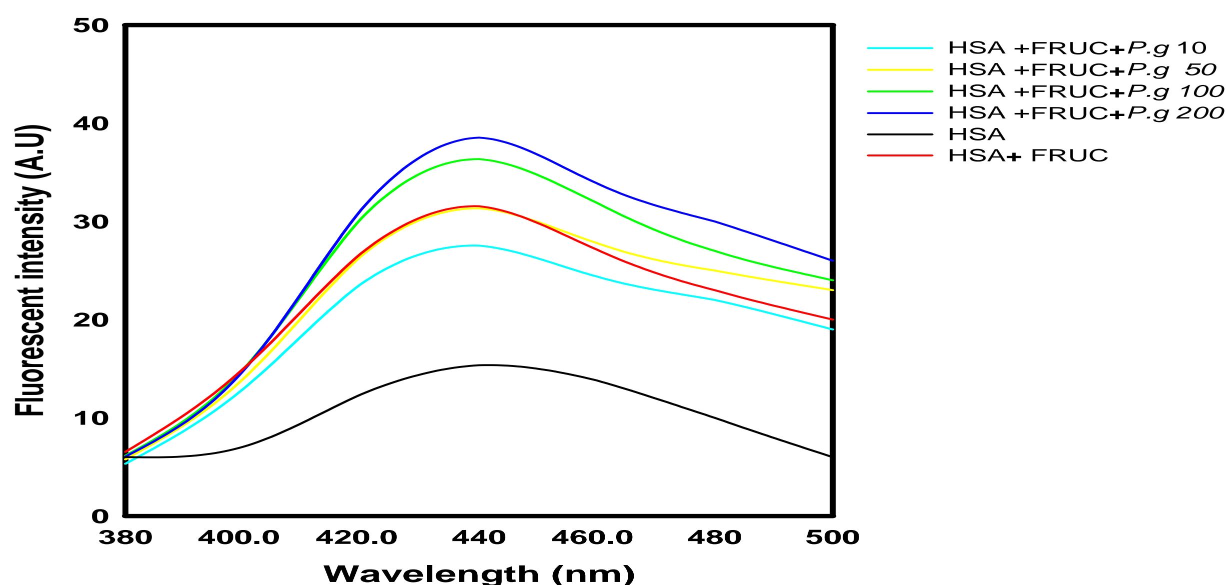

Fig. 2: AGEs specific fluorescence spectra of native, glycated and Aged garlic extract treated glycated HSA samples.

Protein bound Carbonyl Groups

Glycation of HSA with fructose led to an increase in carbonyl content. Further, treatment of glycated HSA sample with Aged garlic extract was associated with decrease in protein bound carbonyl groups in a dose-dependent manner as shown in Fig. 3.

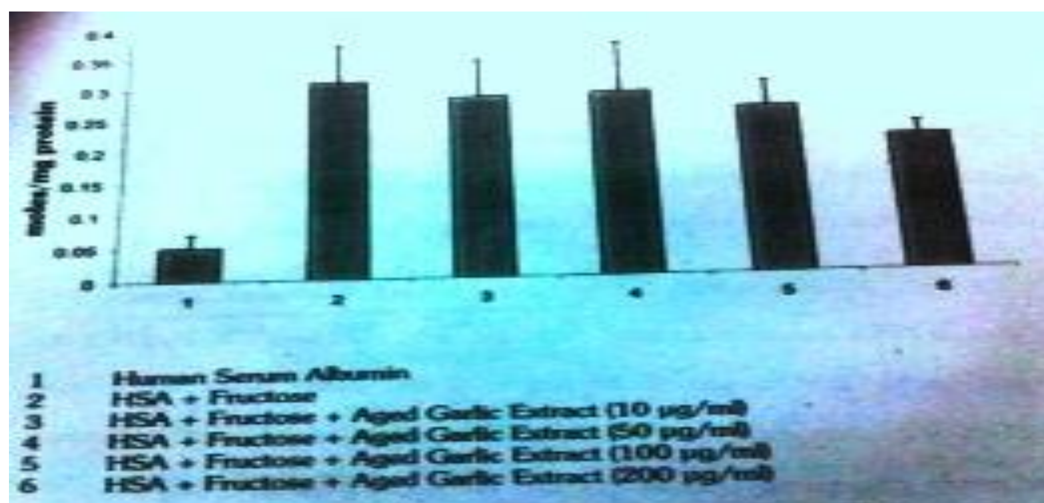

Fig. 3: Determination of protein carbonyls in native, glycated and Aged garlic extract treated glycated HSA samples.

Sodium Dodecyl Sulphate Polyacrylamide Gel (SDS-PAGE) Electrophoresis

Electrophoretic pattern of glycated HSA samples treated with Aged garlic extract at a concentration of 10, 50, 100 and 200µg/ml is shown in figure 4. Aged garlic extract inhibited glycation-induced protein fragmentation and cross-linking causing a reduction in the intensity of the band (Fig.4, lane 4, 5, 6 and 7). This inhibitory effect occurs in a dose-dependent manner with maximum inhibition in samples containing 200µg/ml.

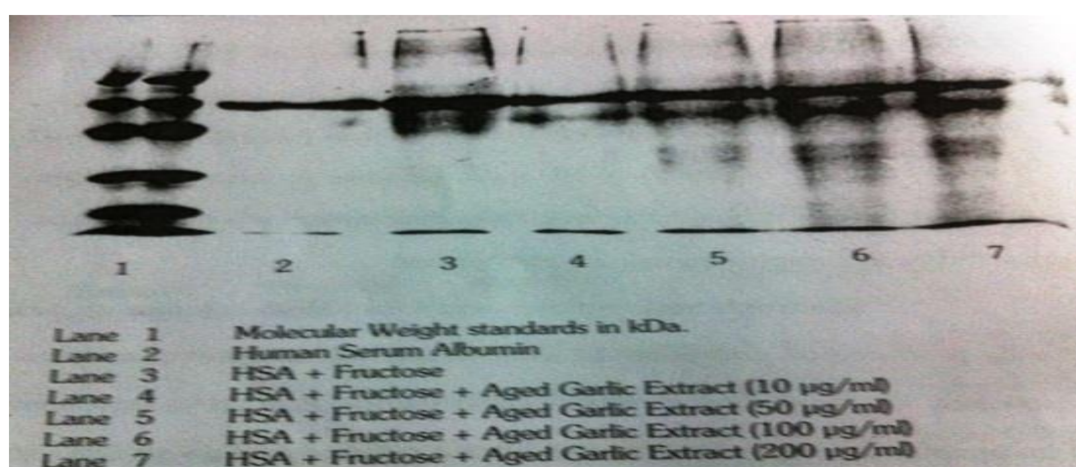

Fig. 4: SDS-PAGE of HSA upon in vitro glycation in the absence and presence of Aged garlic extract.

DISCUSSION

Interaction of proteins with sugars results in structural modification of the former that ultimately lead to formation of AGEs.[1, 2, 20] Higher animals have evolved various strategies to maintain the plasma sugar level thereby avoiding such complications during life-time of an individual. However, there are certain disease situations such as diabetes mellitus when body's regulatory machinery fails to control blood glucose levels and finally ensued in various AGEs related complications.[3, 21]

In the present study we have studied effect of some Aged garlic extract on fructose mediated glycation of model protein Human Serum Albumin.The observed effect of Aged garlic extract on inhibition of fructose mediated modification of HSA could be explained on the basis of anti-oxidative property of garlic extract. Garlic has been reported to be a rich source of allyl sulphides such as diallylsulphide, diallyldisulphide and diallyltrisulphide (formed from allicin). Oxidation processes are important in the formation of many AGEs. It seems garlic components help in inhibition of auto-oxidation of fructose and thereby suppress formation of highly reactive dicarbonyl compounds. Interestingly, there is good correlation with garlic extract mediated inhibition of fructose activity with its ability to form carbonyl compounds. Besides, allyl sulphides, the presence of polyphenols in the garlic extract can also be considered crucial for suppressing carbonyl compound formation and also inhibits protein bound products of the Amadori pattern. Moreover, garlic extract can also decrease ROS formation and thereby inhibits fructose mediated glycation of the HSA.

Similar findings have also been observed by Ahmed et al, where they have observed initial signals of inhibitory effects of aged garlic extract on formation of advanced glycation end products & current study is one more step ahead in exploring antioxidant property of aged garlic extract especially in inhibition of formation of advanced glycation end products.[22]

CONCLUSION

We concluded that herbal source such as garlic extract has the potent inhibitory effect on the protein glycation as it nullified fructose mediated cross-linking, carbonyl formation and AGEs formation as revealed by SDS-PAGE analysis, fluorescence spectroscopy and chemical estimation of carbonyl moiety in HSA.. The observed effect can be attributed to its anti-oxidative as well as free radical scavenging properties.

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