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Allium sativum aqueous extract inhibitory effect on advanced glycation end product

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Abstract

In view of powerful antibiotic, antiviral, antifungal and antioxidant properties of Allium sativum (garlic), the present study was conducted to appraise the effects of garlic extract on glycation end product formation. Different concentrations of inhibitor (I_1 = pure extract, I_2 = 0.5 times, I_3 = 0.1 times diluted) and glucose (G_1 = 50 mM, G_2 = 25 mM, G_3 = 5.5 mM) were used. Thirty two combinations were made and incubated at 37°C for five weeks. Human normal plasma was used as a protein source. Glycation evaluated by TBA (thio-barbituric acid) method, revealed that glycation was maximum in 2nd week of incubation with G₁, while I₂ showed maximum inhibition at 4th week of incubation with all concentrations of glucose. Advanced glycation end products (AGEs), analyzed by ELISA (enzyme linked immunosorbent assay) showed that AGEs formation was maximum at 2nd week with all the glucose concentrations (G_1 , G_2 , G_3), while I_2 showed effective inhibition after 4th week of incubation with G_1 . From results, it is concluded that that garlic extracts has inhibitory effect on advanced glycation end product formation and may be helpful in diabetes and cardiovascular complications reduction.

Key words: Chemopreventive compounds, advanced glycation end products, garlic extract, glucose and antioxidant

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1. Introduction

Allium sativum (garlic) is a member of the family Liliaceae, contains a number of organosulfur compounds (diallyl sulfide, DAS; diallyl disulfide, DADS; S-ethylcysteine, SEC; N-acetylcysteine, NAC) which are considered a potent agents against oxidation and glycation. Alliin is one of the most biologically active compounds in garlic leaf extract and is able to reduces the serum glucose, triglycerides, total lipids, total cholesterol, LDL and VLDLcholesterol level and is thus, beneficialfor uncontrolled diabetes mellitus patients or cardiovascular diseases [1]. Garlic extract inhibits in-vitro formation of advanced glycation end products (AGEPs) as well as glycationderived free radicals due potent antioxidant S-Allylcysteine present in garlic extract [2].

Glycation called also as non-enzymatic glycosylation, is a process in which sugar molecules (glucose or fructose) reacts with lipid and/or protein molecules in the absence of enzymes. Being reducing in nature all types of sugars present in blood are available to react and if this reaction occurs under the action of enzymes

then it termed as glycosylation. Only lysine and arginine residues of the serum albumin protein take part during the glycation reaction. In glycation process, the functions of some biomolecule is disturbed, while in glycosylation this process occur on specific sites of specific molecules which are required for proper functioning of that molecule [3]. Glycation process has ability to make changes in biological structure and functions of serum albumin protein [4-5]. Alteration in serum albumin protein and participation of free radicals in glycation reaction finally lead to the production of AGEP's which damaged tissues by disturbing proteins structure and functions. DNA is also affected by reaction of AGEs and thus, causes transposition by DNA mutations [6]. These are also involved in disturbing some routine products present in cell like nitric oxide production [7]. In recent years AGEs contribution to different diseases such as aging, Alzheimer's disease and diabetes has also received great attention [8].

Antioxidants abundantly present in garlic may help in destroying free radicals that are the major contributor in cell membrane damage, aging process, cancer and heart disease.

In this regard, the present research was conducted to evaluate the inhibitory effect of *Allium sativum* extracts against advanced glycation end products formation.

2. Material and Method

2.1. Sample collection and extraction

The garlic sample was purchased from local market of Faisalabad. The dried and ground sample (5 g) was extracted with water at room temperature for 48 h by shaking, filtered and stored at 4 °C. The Sample was recovered by evaporating the solvent using Rotary Evaporator. The extracts thus obtained were used against advanced glycation end products formation inhibition in three combination (I₁= pure extract, I₂= 0.5 times diluted, I₃= 0.1 times diluted). To study the inhibitory effects, thirtytwo combinations of plasma, glucose and inhibitor were made and placed at 37°C for five weeks [9]. Normal human plasma was collected from civil hospital, Faisalabad, Pakistan. . Samples were withdrawn after 1st, 2nd, 3rd, 4th and 5th week of incubation to perform the experiments for glycation and glycation inhibition.

2.2. Estimation of browning and total protein contents

The sample collected at different time intervals were subjected to browning and protein estimation. For browning 0.1 mL and 4 mL distilled water was taken, mixed and absorbance was measured at 370 nm (CE Cecil 7200) and total protein contents were measured by Biuret method [10].

2.3. Measurement of Glycation level

For glycation measurement, glycated plasma samples were dialyzed against distilled water for 24 h with constant stirring at room temperature to remove the free glucose. The glycation level was measured by TBA (Thiobarbituric acid) method [11]. For non-enzymatic glycation, 0.5 mL plasma and 0.1 mL of 0.01 N NaOH was taken and test tube was kept at 37°C for 30 min. Then, one drop of 1 N HCl and 0.25 mL of oxalic acid (2N) was added. Then tubes were autoclaved for 15 min, cooled and 40% TCA chilled (0.5 mL) was added. Samples were centrifuged for 15 min at 13,000 rpm. After centrifugation, 0.5 mL of TBA (2N) was added in 1 mL of supernatant, incubated at 37 °C for 15 min and absorbance was measured at 443 nm. For enzymatic glycation estimation, the absorbance was measured at 443 nm.

2.4. Advance Glycation End Product (AGEs) detection through ELISA

ELISA was performed by using alkaline phosphate as enzyme and p- nitrophenyl phosphate as a substrate using reported method for the measurement of AGE's [12].

3. Results and Discussion

Plasma incubated with G_1 (50 mM) and buffer showed browning value of 1.061 at 2^{nd} week of incubation

and by further increasing the incubation time browning was decreased and reached to 0.569 after 5th week of incubation. Combination of plasma, G_1 and inhibitors (I_1 , I_2 , I_3) showed browning (1.835) at 5th week of incubation and these values were 1.103 and 1.876 at 1st and 2nd week of incubation, respectively. Among inhibitors, I2 inhibited the browning significantly versus I_1 and I_3 at 37 ⁰C (Fig. 1(A)). Plasma incubated with $G_2(25 \text{ mM})$ showed browning value of 1.040 at 2nd week of incubation, whereas it was decreased by increasing the incubation time and also plasma in combination with G_1 and inhibitors I_1 , I_2 and I_3 showed high browning value of 1.045 at 2nd week of incubation followed by 5^{th} and 2^{nd} week incubation. Among inhibitors, again I_1 inhibited the browning as compared to I_2 and I_3 at 37^{0} C (Fig. 1(B)). Same trend was observed in browning values when plasma incubated with G_3 (5.5 mM) glucose combination (Fig. 1(C)).

3.1. Glycation evaluation by Thiobarbituric Acid Test (TBA)

Plasma incubated with G1 (50 mM) showed glycation value of 4.85 mmole/mole after 3rd week of incubation and the glycation value decreased by increasing the incubation time and at 5th week the glycation level was recorded to be 1.142 mmole/mole. The combination of plasma, G_1 and inhibitors (I_1 , I_2 , I_3) showed maximum value of glycation 13.46 mmole/mole after 2nd week of incubation, whereas this values was 7.12 mole/mole at 3rd week of incubation and so on. On the other hand, I₂ showed maximum inhibition (1.121 mmole/mole) after 4th week, whereas I_1 and I_3 showed lower inhibition (Fig. 2(A)). Plasma incubated with G₂ (25 mM) showed maximum level of glycation 6.45 mmole/mole at 3rd week of incubation, while decreased to 1.167 mmole/mole after 4th week and combination of plasma, G₂ and inhibitors I₁, I₂, I₃ showed maximum level of glycation 4.73 mmole/mole at 3rd week of incubation, whereas these values were 7.419 and 3.02 mmole/mole after 1st week and 2nd week of incubation, respectively. In the presence of G_2 (25 mM), I_2 showed maximum inhibition of 1.07 mmole/mole after 3^{rd} week at 37 ^oC (Fig. 2(B)) and similar trend was observed for plasma incubated with $G_3(5.5 \text{ mM})$, as can be seen in Fig. 2(C).

3.2. Advance glycation end products (AGEs) detection through ELISA

The plasma incubated with G_1 (50 mM) and buffer showed maximum production of AGE's value of 0.091 after 2^{nd} week of incubation, while this value decreased to 0.044 at 5th week of incubation. On the other hand, combination of plasma, G_1 and inhibitors (I_1 , I_2 , I_3) showed maximum level of AGEs formation (0.135) at 5th week of incubation, whereas 0.094 and 0.137 at 3rd and 2nd week of incubation, respectively and again I_2 showed maximum inhibition of AGEs formation (Fig. 3(B)). Similar to G_1 , the G_2 and G_3 also showed same trend AGE's inhibition (Fig. 3(B and C)).



Fig. 1. Browning value of samples at 37°C: A-50 mM Glucose (G₁), B-25 mM Glucose (G₂) and C-5.5 mM Glucose (G₃)



Fig. 2. Determination of Glycation by Thiobarbituric Acid at 37° C: A-50 mM Glucose (G₁), B-25 mM Glucose (G₂) and C-5.5 mM Glucose (G₃)



Fig. 3. Determination of AGEs through ELISA at 37° C: A-50 mM Glucose (G₁), B-25 mM Glucose (G₂) and C-5.5 mM Glucose (G₃)

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Heron and Yarnell [13] reported that allicin present in fresh garlic have antifungal, antiviral and antibacterial properties. Fungal skin infections, such as Tinea corporis and Tinea cruris can be treated by Topical application of garlic. Munday et al. [14] revealed that consumption of garlic help in lowering cardiovascular disease progression. Sekia *et al.* [15] reported that genus *Allium* have phytochemicals which have pharmacological potential, such as antitumor, antithrombotic, antimicrobial and hypoglycemic activities.

Anwar and Meki [16] studied that in streptozotocin induced-diabeteic rat; impaired status of antioxidant can effectively be normalized by garlic oil. Diabetes complications as neuropathy, nephropathy and retinopathy can be delayed by garlic oil. Sheikh et al. [17] observed that diabetes leads to protein glycation which in turn affects biochemical activity and structure of proteins. Garlic has a significant (P<0.05) effect in decreasing or inhibiting the albumin glycation reaction and decreasing diabetic complications. Hwang et al. [18] suggested that glycationderived free radicals formation and formation of AGEPs in vitro is inhibited by aged garlic extract (AGE). Aged garlic key component S-Allylcysteine is a potent antioxidant which inhibits the formation of AGEPs. Ou et al. [19] suggest that four garlic derived organosulfur compounds (diallyl disulfide, DADS; diallyl sulfide, DAS; Nacetylcysteine, NAC; S-ethylcysteine, SEC) are potent agents against glycation and oxidation for protecting LDL and they may benefit in preventing complications of diabetes mellitus and cardiovascular disease patients.

4. Conclusions

From present investigation, it is concluded that garlic has ability to inhibit advanced glycation end products formation that ultimately leads to complex diseases (diabetes mellitus/cardiovascular). Different concentrations of garlic extracts were used in combination with glucose for the evaluation of their inhibitory effect on glycation end products formation and results showed that glycation recorded maximum in 2^{nd} week of incubation, whereas it reduced (inhibited) after 4^{th} week of incubation with different combination of glucose. The results of present study showed that garlic extracts has potential to inhibit the advanced glycation end products formation and might be useful in diabetes and cardiovascular complications reduction.

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