www.nclinmed.com
Novelty in Clinical Medicine. 2022; 1(4): 197-203
https://doi.org/10.22034/NCM.2022.352218.1053

Research Article Open Access

The Comparison of Persian Shallot and Garlic Hydroalcoholic Extracts on Albumin Glycation

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Received: 17 July 2022 **Accepted:** 11 August 2022 **e-Published:** 18 August 2022

Abstract

Background: Hyperglycemia in diabetes leads to body protein glycation, consequently altering their structure and function and causing later complications. So, inhibition of protein glycation reaction or breaking this linkage for reducing or improving diabetes complications seems so necessary.
Objectives: In the current study the effect of Persian Shallot and garlic hydroalcholic extracts on the amount of in vitro albumin glycation was compared.
Methods: In this experimental study, the effect of 0.1, 0.2, and 1 gr/dl concentration of Persian shallot and garlic extracts, on inhibition of albumin glycation reaction was compared. The amount of glycation was assayed by TBA (Thiobarbituric Acid).
Results: The Persian shallot and garlic extracts in all concentrations inhibit the albumin glycation reaction and the 1 g/dl concentration was the most effective and the differences were significant ($P<0.05$). The rate of linkage breaking has a direct relation to concentration and the time period of treatment. The observed inhibitory effects showed a significant difference between Persian shallot and garlic extract only at a concentration of 1 g/dl in 48 hours of treatment, which showed that Persian shallot had a greater effect.
Conclusion: The current findings demonstrate that Persian shallot and garlic similarly prevent albumin glycation and also break the linkage between albumin and glucose.

Keywords: Diabetes Mellitus, Persian Shallot, Garlic, Albumin Glycation, in vitro.

Introduction

Diabetes is one of the most common endocrine disorders in the world.[1] Increased glycosylation of proteins is reported in diabetes. The glycosylation reaction takes place spontaneously whenever proteins are exposed to reduced sugars, and its amount depends on the severity of hyperglycemia and its duration in the body. The formation of glycosylated proteins changes the structure and biochemical activity of proteins and leads to the production of free radicals through the autoxidation of glucose. Free radicals can damage lipids, proteins, and nucleotides and possibly cause tissue damage in diabetes.[2] It is possible that during hyperglycemia, a direct reaction (Maillard reaction) takes place between proteins and sugars. In fact, the non-enzymatic reaction in which sugars are attached to the protein chain of lipids or nucleotides is called glycosylation. The formation of glycosylated proteins plays a role in the onset of diabetes, kidney failure, and Alzheimer.[3]

One of the important proteins in the body that undergoes non-enzymatic glycosylation is serum albumin. Albumin constitutes about 60% of plasma proteins and has important roles such as maintaining oncotic pressure and transporting endogenous and exogenous biomolecules.[4]

So special attention has been paid to compounds that can inhibit glycosylation.[3] These compounds can probably inhibit the formation of glycosylated proteins by blocking

carbonyl groups in reduced sugars.[5] New drugs have been released that can break the cross-links of glycosylated proteins and play a role in improving diabetes disorders. Antioxidants have a protective effect against free radicals caused by glycosylation.[6]

Epidemiological studies show that the consumption of fruits and vegetables reduces the complications of chronic diseases such as cardiovascular diseases, cancer, and diabetes. Phytochemicals such as flavonoids, phenols, and organosulfur compounds are the most important effective compounds found in fruits and vegetables, which have antioxidant effects.[7]

For a long time, due to the ease of access and in most cases, fewer side effects, medicinal plants have had a special place in medical science for the treatment of common human diseases, especially those diseases with a metabolic nature such as diabetes. Also, the investigation of the effectiveness of medicinal plants or natural substances derived from them with hypoglycemic properties in order to reduce tissue complications caused by diabetes has dramatically increased in recent years. Recent studies have proven antioxidant and antiglycosylation properties in medicinal plants.[8]

The onion family (Alliaceae), which includes garlic and shallots, has been used for food and medicine since about 5 thousand years ago, and in the last 40 years has received more attention.[8] Garlic has anti-bacterial, anti-viral, anti-atherosclerosis, anti-inflammatory, anti-cancer, and anti-diabetic effects. Garlic has long been used as a medicine to treat many diseases and today it is available in different forms in the market.[9] Garlic has an inhibitory effect on the formation of glycosylated proteins in the laboratory environment. There is no information about the effect of garlic in preventing the formation of glycosylated proteins in vivo.[10]

Allium hirtifolium or Persian shallot is a traditional Iranian herb and spice that ancient Iranians knew about the medical benefits of this plant and used to treat diseases such as inflammation and rheumatism.[11,12] *Allium hirtifolium* belongs to the Alliaceae family. It is native to Iran and is known as Persian shallot in Iran. This plant belongs to the kingdom of Plantae, clade of Angiosperms, clade of Monocots, order of Asparagales, family of Alliaceae, genus of Allium, species of *A. hirtifolium*. Shallot contains organosulfur compounds, flavonoids and phenols, which are richer in terms of flavonoids than garlic.[11,12]

Objectives

The aim of this study is to compare the effect of hydroalcoholic extracts of Persian shallot and garlic on inhibition of in vitro albumin glycation and albumin-glucose bond breaking.

Methods

Hydroalcoholic extract of Persian shallot and garlic

The following method was used to prepare the hydroalcoholic extract; 100 grams of fresh and homogenized shallots were incubated with 400 ml of a mixture of water and ethanol (25:75) for 48 hours. Then it was filtered, evaporated, and dried in a rotator evaporator under reduced pressure.[13] Then, the concentrations of 0.1, 0.2, 1 g/dl were prepared from this powder. This process was also performed separately for garlic.

Albumin glycosylation reaction

One ml of 30% glucose solution was added to 1 ml of 5g/dl albumin solution. To prevent environmental pollution, gentamicin with a concentration of 0.2 g/l was added in 0.01 M phosphate buffer with pH=7.4 and 3 mM sodium azide and it was incubated for 72 hours at room temperature. After the incubation, it was dialyzed in phosphate buffer (dialysis bag prepared in 10 mmol/L EDTA).[14]

Measurement of albumin glycosylation

TBA (thiobarbituric acid) test was used to confirm whether albumin is glycated or not. In this test, thiobarbituric acid reagent is combined with the products resulting from the albumin glycosylation reaction and produces a substance with maximum absorption at 443 nm.[15]

The effect of Persian shallot and garlic extracts on albumin and glucose bond breaking

Here, the albumin glycosylation reaction was performed, then different concentrations of Persian shallot and garlic

extract (0.1, 0.2, 1 g/dl) were added to them. After 24, 48, 72 hours of treatment, the albumin and glucose bond breaking were measured by TBA method.

The effect of Persian shallot and garlic extracts on inhibition of albumin glycosylation reaction

0.1 ml of different concentrations of Persian shallot and garlic (0.1, 0.2 and 1 gr/dl) was added to 1 ml of 5% albumin solution and 1 ml of glucose 30 g/l (in phosphate buffer solution and gentamicin) (the extract and glucose were added to albumin at the same time) and incubated at laboratory temperature for 72 hours.

TBA test was used to determine the effect of each concentration of Persian shallot and garlic extracts. The decrease in the amount of absorption compared to the control group shows the effect on the albumin glycation reaction.

Control group

Shallot or garlic extract was not added to the control solution (albumin+glucose), and the absorbance was measured at the wavelength of 443 nm.

Statistical analysis

The continuous variables were expressed as the mean ± SD. Data was analysed using one-way analysis of variance and then Tukey's test as well as Repeated Measure. All statistical analyses were performed with SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). A “P-value” less than 0.05 was considered significant.

Results

The effect of Persian shallot and garlic extracts on inhibition of albumin glycation reaction

According to Figure 1, Persian shallot and garlic extracts had an inhibitory effect in all concentrations, and this inhibitory effect was dependent on the concentration. The inhibitory effects of 0.2 and 1 g/dl were significant compared to the control group ($P<0.05$). The highest inhibitory effect was related to the concentration of 1 g/dl in Persian shallot and garlic extract. As shown in Figure 1, the observed inhibitory effects do not show a significant difference between Persian shallot and garlic extracts.

The effect of Persian shallot and garlic extracts on albumin and glucose bond breaking after 24 hours of treatment

During 24 hours of treatment of glycosylated albumin with different concentrations of Persian shallot and garlic extract, only the concentration of 1 g/dl of Persian shallot and garlic extracts significantly reduced the amount of sugar albumin and broke the bond of glycosylated albumin compared to the control group. The concentrations of 0.1 and 0.2 g/dl of Persian shallot and garlic extracts did not have much effect. On the other hand, the concentration of 1 g/dl of Persian shallot extract significantly reduced glycosylated albumin compared to groups one and two ($P<0.05$), but this effect was not observed in garlic (Figure 2).

The effect of Persian shallot and garlic extracts on albumin and glucose bond breaking after 48 hours of treatment

Treatment with concentrations of 0.2 and 1 g/dl of Persian shallot and garlic extracts for 48 hours significantly reduced glycosylated albumin and broke the bond between glucose and albumin ($P<0.05$), which was the most effective at 1 g/dl concentration (Figure 3). While 0.1 concentration of Persian shallot and garlic extract did not show any significant effect. The observed inhibitory effects show a significant difference between Persian shallot and garlic extracts only at a concentration of 1 g/dl, which shows that shallot had a greater effect.

The effect of Persian shallot and garlic extracts on albumin and glucose bond breaking after 72 hours of treatment

Treatment with different concentrations of Persian shallot and garlic extracts for 72 hours significantly reduced glycosylated albumin ($P<0.05$). This effect was dependent on the concentration and the concentration of 1 g/dl showed the highest effect which is significant even compared to group one ($P<0.05$) (Figure 4). The observed inhibitory effects do not show a significant difference between Persian shallot and garlic extracts.

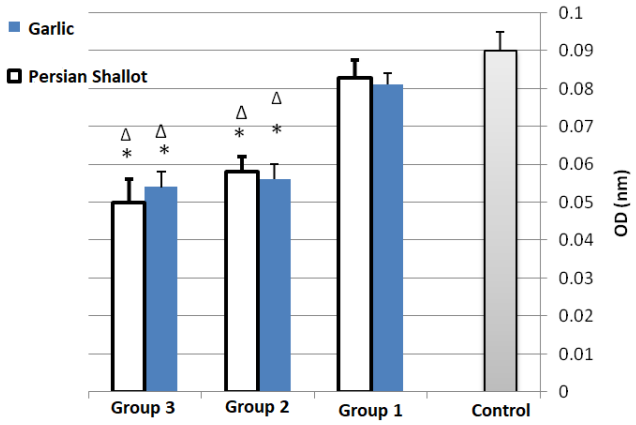

Figure 1. The effect of different concentrations of Persian shallot and garlic extract on the inhibition of albumin glycosylation reaction

(*) significant difference with group one. (Δ) significant difference with group two

Control group: albumin and glucose without extract

Group one: 0.1 g/dl of Persian shallot and garlic extract

Group two: 0.2 g/dl of Persian shallot and garlic extract

Group three: 1 g/dl of Persian shallot and garlic extract

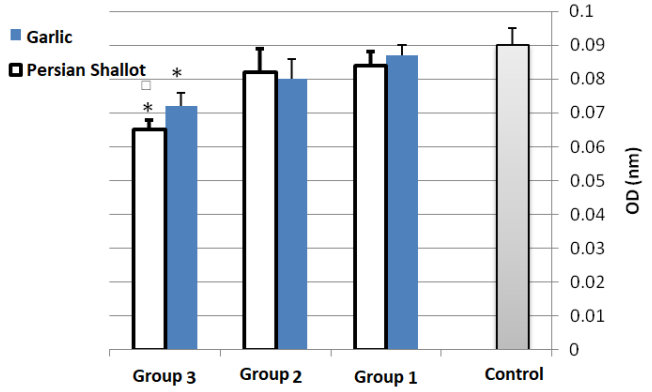

Figure 2. The effect of different concentrations of Persian shallot and garlic extract on the breaking of albumin and glucose bond (24 hours of treatment)

(*): significant difference with group one. (□): significant difference with groups two and three.

Control group: albumin and glucose without extract

Group one: 0.1 g/dl of Persian shallot and garlic extract

Group two: 0.2 g/dl of Persian shallot and garlic extract

Group three: 1 g/dl of Persian shallot and garlic extract

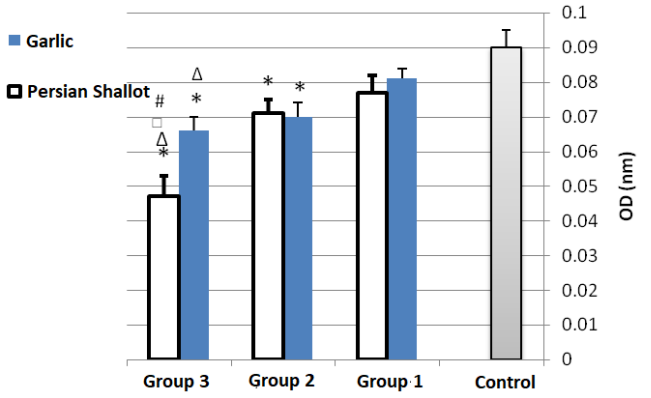

Figure 3. The effect of different concentrations of Persian shallot and garlic extract on the breaking of albumin and glucose bond (48 hours of treatment)

(*): significant difference with group one. (□): significant difference with three. (Δ): significant difference with group two. (#): significant difference with garlic in the same group.

Control group: albumin and glucose without extract

Group one: 0.1 g/dl of Persian shallot and garlic extract

Group two: 0.2 g/dl of Persian shallot and garlic extract

Group three: 1 g/dl of Persian shallot and garlic extract

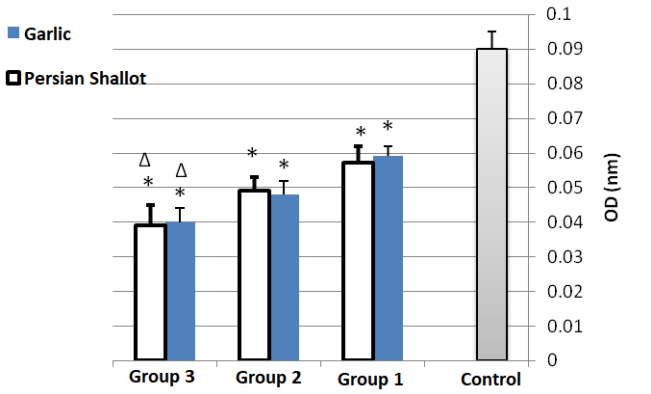

Figure 4. The effect of different concentrations of Persian shallot and garlic extract on the breaking of albumin and glucose bond (72 hours of treatment)

(*): significant difference with group one. (Δ): significant difference with group two.

Control group: albumin and glucose without extract

Group one: 0.1 g/dl of Iranian shallot and garlic extract

Group two: 0.2 g/dl of Iranian shallot and garlic extract

Group three: 1 g/dl of Iranian shallot and garlic extract

Discussion

In the present study, the effect of Persian shallot and garlic extracts at concentrations of 0.1, 0.2 and 1 g/dl on the inhibition of albumin glycosylation reaction (adding extract and glucose to albumin at the same time) was investigated. Persian shallot and garlic extracts had an inhibitory effect in all concentrations, and the highest effect was related to the concentration of 0.2 and 1 g/dl. A direct relationship was observed between the concentration of the extract and the inhibition of the reaction so that the inhibitory effect was greater with the increase in concentration. Of course, the observed inhibitory effects do not show a significant difference between Persian shallot and garlic extracts.

According to our knowledge, there are not many published studies in this field, only a study conducted by Hosseini et al. in the Department of Clinical Biochemistry at Rafsanjan University of Medical Sciences, which is somewhat in line with the current findings. It was shown that Abu Jahl watermelon extract has an inhibitory effect in all concentrations (0.1, 0.2, 0.5 and 1 g/dl), and the highest effect was related to the concentration of 0.2 g/dl, but no clear relationship was observed between the extract concentration and reaction inhibition.[16]

In another part of the present study, the effect of Persian shallot and garlic extracts at concentrations of 0.1, 0.2 and 1 g/dl on breaking the bond of albumin and glucose was investigated. As the treatment time increased (24 hours, 48 hours and 72 hours), the extracts broke more bonds in glycosylated albumin, which is probably due to the increase in the exposure time of the extracts with the albumin-glucose bond, and this point indicates that if the consumption of the Persian shallots and garlic continue, the effect on the complications of diabetes will increase. Also, as shown in Figures 2, 3, and 4, at all times of treatment, the concentration of 1 g/dl had the greatest effect on breaking the albumin-glucose bond. It was determined that bond breaking in the glycosylated albumin depends on time and concentration. However, in Hosseini et al.'s study on Abu Jahl watermelon, this effect was shown to be dependent only on the treatment time.[16]

The observed inhibitory effects do not show a significant difference between Persian shallot and garlic extracts and the effects are somewhat similar. Except for the treatment time of 48 hours and only at the concentration of 1 g/dl, Persian shallot had a significantly more positive effect. The possible reason is the existence of organosulfur compounds, flavonoids and phenols, which Persian shallots are richer in terms of flavonoids than garlic.[12]

In a study conducted by Safari et al., on some herbal products, volatile oils such as thymol, geraniol, linalool, and limonene caused a decrease in non-enzymatic glycosylation reaction (in vitro).[17] In another study, it was shown that turmeric, cardamom and ginger can reduce the non-enzymatic glycation of albumin in vitro.[18] All these studies are consistent with the findings of the present study. Considering that Persian shallot and garlic extracts contain flavonoid and organosulfur compounds. It seems that its hypoglycemic effects are due to these components.[12]

Nowadays, a lot of attention has been paid to inhibitors of glycation reactions due to their therapeutic potential. Anti-glycation compounds probably prevent the formation of advanced sugar products by blocking carbonyl groups on reduced sugars, Amadori products and 3-deoxyglucosones. Probably, in the present study, extracts of Persian shallot and garlic have acted in this way. Recently, drugs have been found that break the cross-links of advanced sugar products and reduce the complications of diabetes.[17,19] In the current study, Persian shallot and garlic extracts showed such an effect, which requires further studies to understand the possible mechanisms more precisely.

In another study, the effect of different flavonoids on the reaction of non-enzymatic glycosylation of proteins was investigated. Flavonoids, rutin, kaempferol, quercetin, apigenin, and naringenin reduced the non-enzymatic glycation of albumin, hemoglobin, and insulin in vitro.[20] Also, Delers et al., showed that some polyphenol compounds found in plants are able to reduce the glycation of proteins in the blood, such as haptoglobin.[21] In a study, it was shown that S-allyl cysteine present in garlic is the main and effective anti-diabetes compound.[22] In addition to these, other studies have been conducted on the anti-diabetic effects of Persian shallots. The results of Hosseini et al.'s study showed that Persian shallot extract

is effective in reducing blood sugar and improving the profile of enzymes involved in blood sugar metabolism in diabetic mice.[23,24]

Conclusions

In the present study, Persian shallot and garlic extract demonstrate hypoglycemic and antiglycemic effects both by preventing the non-enzymatic glycosilation reaction of albumin and by breaking the bond between albumin and glucose, which can be attributed to the presence of some compounds present in these plants.

Acknowledgment

We are very grateful to the honorable research vice president of Hamedan University of Medical Sciences and the student research committee who supported this research. Also, we would like to sincerely thank Mrs. Zadkhosh, Mr. Shaisheta, and Dr. Ghafouri for their cooperation in carrying out this project at the Faculty of Pharmacy of Hamedan University of Medical Sciences, Hamadan, Iran.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Thiobarbituric acid: TBA.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

None.

Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

Institutional Review Board approval was obtained.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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