

# Comparison of the effects of Persian shallot and garlic hydroalcoholic extracts on albumin glycation

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## Abstract

**Background:** Hyperglycemia in diabetes leads to body protein glycation, altering its structure and function and causing later complications. Therefore, inhibition of the protein glycation reaction or breaking this linkage is crucial for reducing diabetes complications.

**Objectives:** This study aimed to compare the impact of hydroalcoholic extracts of Persian shallot and garlic on the level of *in vitro* albumin glycation.

**Methods:** This study aimed to examine the inhibitory effects of Persian shallot and garlic extracts at concentrations of 0.1, 0.2, and 1 g/dL on albumin glycation reactions. The assessment of glycation levels was conducted using thiobarbituric acid (TBA).

**Results:** Both Persian shallot and garlic extracts significantly inhibited the glycation reaction of albumin at all concentrations. Notably, the concentration of 1 g/dL exhibited the highest efficacy, displaying statistically significant variances ( $P < 0.05$ ). The extent of linkage breaking is directly correlated with the concentration of the extracts and the duration of treatment. Furthermore, when administered at a concentration of 1 g/dL, the inhibitory effects of Persian shallot and garlic extracts were significantly different 48 hours after treatment, showing that Persian shallot had a greater impact.

**Conclusion:** The present study provides evidence that both Persian shallot and garlic possess the ability to inhibit albumin glycation and break the linkage between albumin and glucose.

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

## Introduction

Diabetes is a prevalent endocrine disorder worldwide, ranking among the most common.<sup>1</sup> In individuals with diabetes, there is an observed increase in the glycosylation of proteins. When proteins come into contact with reduced sugars, a glycosylation process occurs, which is affected by the degree and duration of hyperglycemia in the body. The formation of glycosylated proteins brings about structural and biochemical changes, ultimately leading to the generation of free radicals through glucose autoxidation. These free radicals have the potential to

harm lipids, proteins, and nucleotides, thereby contributing to tissue damage in individuals with diabetes.<sup>2</sup>

It is plausible that a direct chemical reaction known as the Maillard reaction occurs between proteins and sugars during a state of high blood sugar levels, also known as hyperglycemia. This non-enzymatic reaction, called glycosylation, involves the attachment of sugars to the protein chains of lipids or nucleotides. The formation of glycosylated proteins is implicated in the development of diabetes, kidney failure, and Alzheimer's disease.<sup>3</sup> One

notable protein in the body that undergoes non-enzymatic glycosylation is serum albumin. Constituting approximately 60% of plasma proteins, albumin plays crucial roles in maintaining oncotic pressure and facilitating the transport of both endogenous and exogenous biomolecules.<sup>4</sup>

Considerable emphasis has been placed on compounds with the ability to impede glycosylation.<sup>3</sup> These compounds likely hinder the creation of glycosylated proteins by obstructing carbonyl groups in reduced sugars.<sup>5</sup> Recently, novel medications have been introduced that can break the cross-links of glycosylated proteins, thereby contributing to the amelioration of diabetes-related conditions. Antioxidants exhibit a protective effect against the detrimental impact of free radicals induced by glycosylation.<sup>6</sup>

Epidemiological research has demonstrated that the intake of fruits and vegetables can mitigate the complexities associated with chronic ailments like cardiovascular diseases, cancer, and diabetes. Phytochemicals, including flavonoids, phenols, and organosulfur compounds, are the main active components found in fruits and vegetables, showing strong antioxidant properties.<sup>7</sup>

For a considerable period, medicinal plants have held a significant position in the field of medical science for treating common human ailments, particularly metabolic diseases like diabetes, owing to their accessibility and minimal side effects. In recent years, there has been a substantial increase in the exploration of medicinal plants and their derived natural substances with hypoglycemic properties, aiming to mitigate tissue complications caused by diabetes. Recent research has successfully demonstrated the antioxidant and anti-glycosylation attributes of medicinal plants. Among them, the Alliaceae family, encompassing garlic and shallots, has been utilized for both culinary and medicinal purposes for approximately 5,000 years, gaining heightened attention in the past four decades.<sup>8</sup> Garlic exhibits various beneficial effects, including antibacterial, antiviral, anti-atherosclerosis, anti-inflammatory, anti-cancer, and anti-diabetic properties. Throughout history, garlic has been employed as a medicinal remedy for numerous ailments and is

presently available in diverse forms on the market.<sup>9</sup> Garlic exhibits an inhibitory impact on the creation of glycosylated proteins in a laboratory setting. However, there is insufficient data available regarding the potential of garlic to hinder the formation of glycosylated proteins *in vivo*.<sup>10</sup>

Ancient Iranians were well aware of the medicinal properties of *Allium hirtifolium*, also known as Persian shallot, which is a traditional herb and spice in Iran. This plant, belonging to the Alliaceae family, is native to Iran and has been used for treating various ailments such as inflammation and rheumatism. *Allium hirtifolium* falls under the kingdom Plantae, clade Angiosperms, clade Monocots, order Asparagales, family Alliaceae, genus *Allium*, and species *A. hirtifolium*. Persian shallot, unlike garlic, is particularly abundant in flavonoids, along with organosulfur compounds and phenols.<sup>11,12</sup>

## Objectives

This study aimed to evaluate the effects of hydroalcoholic extracts of Persian shallot and garlic on the inhibition of *in vitro* albumin glycation and the breaking of albumin-glucose bonds.

## Methods

### Preparation of hydroalcoholic extract from Persian shallot and garlic

To obtain the hydroalcoholic extract, the following procedure was employed: Initially, 100 grams of fresh shallots were finely ground and combined with a mixture of water and ethanol (25:75) in a total volume of 400 ml. The mixture was allowed to incubate for 48 hours. Subsequently, it underwent filtration, evaporation, and drying using a rotary evaporator under reduced pressure.<sup>13</sup> This resulted in the formation of a powdered extract. To create different concentrations, namely 0.1, 0.2, and 1 g/dL, the powder was further diluted accordingly. The same process was carried out independently for garlic as well.

### Albumin glycosylation reaction

A 30% glucose solution, measuring one mL, was combined with a one mL solution of albumin with a

concentration of 5 g/dL. To avoid causing harm to the environment, a gentamicin solution with a concentration of 0.2 g/L was added to a 0.01 M phosphate buffer with a pH of 7.4 and 3 mM sodium azide. The mixture was then incubated at room temperature for 72 hours. Following the incubation period, the solution was subjected to dialysis using phosphate buffer and a dialysis bag prepared with 10 mmol/L EDTA.<sup>14</sup>

#### Measurement of albumin glycosylation

The utilization of the TBA (thiobarbituric acid) test aimed to verify the presence of glycated albumin. This test includes combining the thiobarbituric acid reagent with the byproducts produced by the reaction of albumin with glucose, resulting in the creation of a compound with maximum absorption at 443 nm.<sup>15</sup>

#### Effect of Persian shallot and garlic extracts on albumin and glucose bond break

The albumin glycosylation process was carried out, followed by the addition of various concentrations (0.1, 0.2, and 1 g/dL) of Persian shallot and garlic extract. Subsequently, albumin and glucose bond breaking were assessed using the TBA method after 24, 48, and 72 hours of treatment.

#### Effect of Persian shallot and garlic extracts on inhibition of albumin glycosylation reactions

Persian shallot and garlic extracts (0.1, 0.2, and 1 g/dL) were mixed with 0.1 mL of 5% albumin solution and 1 mL of glucose (30 g/L) in phosphate buffer solution and gentamicin. The mixture, consisting of the extract and glucose, was added to the albumin simultaneously. The resulting solution was then incubated at laboratory temperature for 72 hours. To determine the impact of each concentration of Persian shallot and garlic extracts on the albumin glycation reaction, the TBA test was employed. The reduction in absorption compared to the control group indicates the influence of the extracts on the albumin glycation reaction.

#### Control group

No shallot or garlic extract was included in the control solution (albumin and glucose), and the absorbance was determined at a wavelength of 443 nm.

#### Statistical analysis

The continuous variables were expressed as the mean  $\pm$  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

##### Effect of Persian shallot and garlic extracts on inhibition of albumin glycation reaction

Based on Figure 1, it can be observed that both Persian shallot and garlic extracts exhibited inhibitory effects across all concentrations, with the intensity of inhibition being dependent on the concentration. Notably, the inhibitory effects of 0.2 and 1 g/dL were found to be significantly different from the control group ( $P < 0.05$ ). The highest level of inhibition was observed at a concentration of 1 g/dL for both Persian shallot and garlic extracts. Furthermore, Figure 1 indicates that there is no significant difference in the observed inhibitory effects between Persian shallot and garlic extracts.

##### Effect of extracts derived from Persian shallot and garlic on the breaking of albumin and glucose bonds following a 24-hour treatment

The effect of different concentrations of Persian shallot and garlic extracts on sugar albumin levels and the bond of glycosylated albumin was investigated over 24 hours. When compared to the control group, only the concentration of 1 g/dL Persian shallot and garlic extracts showed a significant reduction in both sugar albumin levels and the glycosylated albumin bond. Conversely, concentrations of 0.1 and 0.2 g/dL of Persian shallot and garlic extracts had minimal effect. Furthermore, a concentration of 1 g/dL of Persian shallot extract showed a significant reduction in glycosylated albumin compared to groups one and two ( $P < 0.05$ ), although garlic did not [Figure 2].

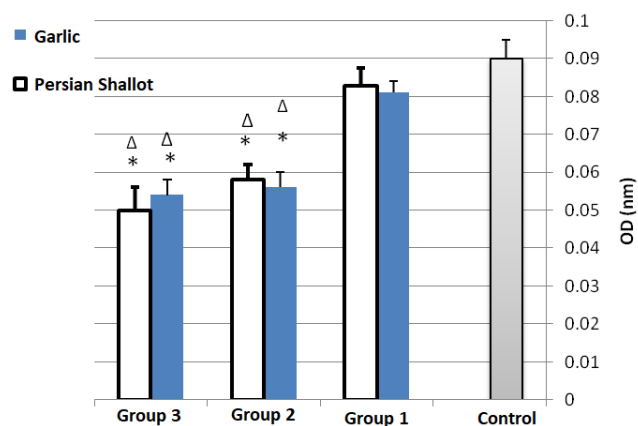
##### Effect of extracts derived from Persian shallot and garlic on the breaking of albumin and glucose bonds following a 48-hour treatment period

Treatment with Persian shallot and garlic extracts at

concentrations of 0.2 and 1 g/dl for 48 hours significantly reduced glycosylated albumin and broke the bond between glucose and albumin ( $P < 0.05$ ), with the 1 g/dl concentration being the most effective [Figure 3], while the 0.1 concentration had no significant effect. Interestingly, the inhibitory effects were only significantly different between Persian shallot and garlic extracts at the 1 g/dL concentration, suggesting that shallot had a greater impact.

**Effect of extracts derived from Persian shallot and garlic on the breaking of albumin and glucose bonds following a 72-hour treatment period**

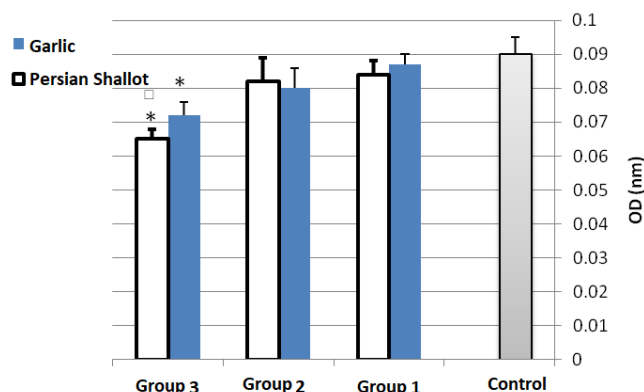
The glycosylated albumin levels were significantly reduced after a 72-hour treatment with varying concentrations of Persian shallot and garlic extracts ( $P < 0.05$ ). The extent of this reduction was found to be dependent on the concentration used, with the 1 g/dL concentration exhibiting the highest effect. Notably, this concentration showed a significant reduction even when compared to Group 1 ( $P < 0.05$ ) [Figure 4]. Interestingly, there was no significant difference observed in the inhibitory effects between the extracts of Persian shallot and garlic.



**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

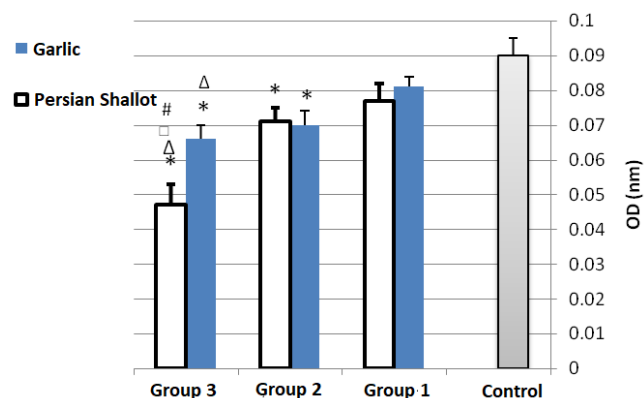
- Control group: albumin and glucose without extract
- Group one: 0.1 g/dl of Persian shallot and garlic extract
- Group two: 0.2 g/dl of Persian shallot and garlic extract
- Group three: 1 g/dl of Persian shallot and garlic extract



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

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

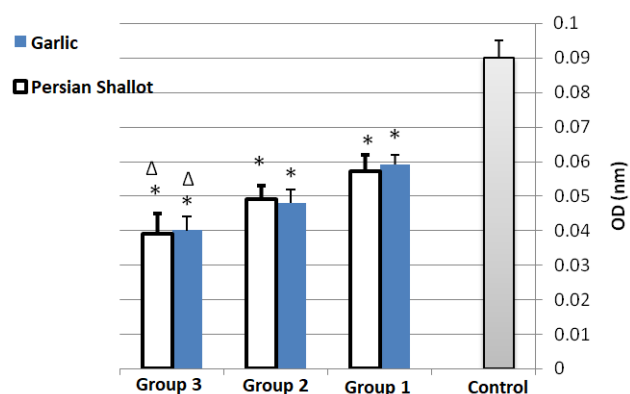
- Control group: albumin and glucose without extract
- Group one: 0.1 g/dl of Persian shallot and garlic extract
- Group two: 0.2 g/dl of Persian shallot and garlic extract
- Group three: 1 g/dl of Persian shallot and garlic extract



**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

The current investigation aimed to examine the impact of Persian shallot and garlic extracts on the inhibition of albumin glycosylation reactions. The extracts were tested at concentrations of 0.1, 0.2, and 1 g/dL. Results indicated that both Persian shallot and garlic extracts exhibited inhibitory effects across all concentrations. Notably, the highest inhibitory effect was observed at concentrations of 0.2 and 1 g/dL.

A clear correlation was noted between the concentration of the extract and the inhibition of the reaction, whereby the inhibitory impact intensified as the concentration increased. Importantly, there was no notable distinction in the observed inhibitory effects between Persian shallot and garlic extracts.

To the best of our understanding, published research is scarce in this particular field. Only one study, conducted by Hosseini et al. at the Department of Clinical Biochemistry at Rafsanjan University of Medical Sciences, has been published. This study revealed that Abu Jahl watermelon extract exhibited inhibitory properties across all tested concentrations (0.1, 0.2, 0.5, and 1 g/dL), with the highest level of inhibition observed at a concentration of 0.2 g/dL. However, no definitive correlation was observed between the concentration of the extract and the extent of

inhibition of the reaction.<sup>16</sup>

In a separate phase of the current study, the effect of Persian shallot and garlic extracts on the breaking bond between albumin and glucose was investigated at concentrations of 0.1, 0.2, and 1 g/dL. As the duration of treatment increased (24 hours, 48 hours, and 72 hours), the extracts exhibited a greater ability to break the bonds within glycosylated albumin. This can be attributed to the prolonged exposure of the extracts to the albumin-glucose bond. This discovery suggests that the consumption of Persian shallots and garlic could potentially amplify the effects of diabetes-related complications.

Furthermore, as depicted in Figures 2, 3, and 4, the concentration of 1 g/dL demonstrated the most significant impact on breaking the albumin-glucose bond throughout all treatment durations. It was determined that the breaking of glycosylated albumin bonds is contingent upon both time and concentration. However, in Hosseini et al.'s investigation of Abu Jahl watermelon, this effect was found to be solely dependent on the duration of treatment.<sup>16</sup>

There was no significant distinction in the inhibitory effects observed between extracts of Persian shallot and garlic, as the effects are relatively similar. However, when considering a treatment time of 48 hours and a concentration of 1 g/dL, Persian shallot exhibits a notably more favorable impact. This discrepancy can be attributed to the higher abundance of organosulfur compounds, flavonoids, and phenols in Persian shallots, particularly in terms of flavonoid content when compared to garlic.

Safari et al. conducted a study on several herbal products and discovered that the presence of volatile oils such as thymol, geraniol, linalool, and limonene reduced non-enzymatic glycosylation reactions (in vitro).<sup>17</sup> Another study revealed that turmeric, cardamom, and ginger possess the ability to reduce the non-enzymatic glycation of albumin in vitro.<sup>18</sup> These studies support the current study by confirming the presence of flavonoid and organosulfur compounds in Persian shallot and garlic extracts. These constituents are believed to be responsible for the hypoglycemic properties exhibited by these extracts.<sup>12</sup>

In recent times, there has been a significant focus on

inhibitors of glycation reactions due to their potential therapeutic applications. Anti-glycation compounds are thought to inhibit the formation of advanced sugar products by blocking carbonyl groups on reduced sugars, Amadori products, and 3-deoxyglucosones. Likely, the extracts of Persian shallot and garlic employed in the current investigation have operated similarly. Notably, new drugs have been identified that break the cross-links of advanced sugar products and mitigate the complications associated with diabetes. In the present study, the extracts of Persian shallot and garlic exhibited an effect, thereby necessitating further exploration to gain a more comprehensive understanding of the underlying mechanisms involved.<sup>19</sup>

A separate investigation examined the impact of various flavonoids on the non-enzymatic glycosylation reactions of proteins. Flavonoids rutin, kaempferol, quercetin, apigenin, and naringenin were reported to reduce the non-enzymatic glycation of albumin, hemoglobin, and insulin in laboratory conditions.<sup>20</sup> Furthermore, Delers et al. demonstrated that certain polyphenol compounds present in plants possess the ability to diminish protein glycation in the bloodstream, including haptoglobin.<sup>21</sup>

A research investigation revealed that S-allyl cysteine, which is found in garlic, serves as the primary and most potent anti-diabetic compound.<sup>22</sup> Furthermore, various other studies have been carried out to explore the anti-diabetic properties of Persian shallots. The findings from Hosseini et al.'s study demonstrated that the extract of Persian shallots effectively lowers blood sugar levels and enhances the activity of enzymes involved in blood sugar metabolism in diabetic mice.<sup>23,24</sup>

## Conclusions

According to the current study, Persian shallot and garlic extract have hypoglycemic and antiglycemic effects by inhibiting non-enzymatic glycosilation of albumin and breaking the bond between albumin and glucose, which can be associated with the presence of certain chemicals found in these plants.

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## 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.

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## 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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