



Effects of ethanolic extract of *Artemisia Sieberi* Besser on DNA glycation of glucose: Possible antidiabetic mechanism

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Abstract

Background: DNA glycation damages DNA by inducing strand breaks, mutations, and ultimately changes in gene expression, which is considered a main factor in the pathogenesis of diabetes and its complications. Therefore, antiglycation agents have become the focus of recent research for preventing and alleviating diabetes complications. According to the reported antidiabetic effects of *Artemisia sieberi* (*A. sieberi*) leaf extract, this study aimed to determine the effect of the ethanolic extract of *A. sieberi* on glucose-mediated DNA glycation for the first time.

Methods: DNA was incubated with glucose in the presence or absence of *A. sieberi* for 4 weeks. The inhibitory or facilitatory effects of *A. sieberi* on DNA structural changes were studied by various techniques. These techniques included UV-Vis, fluorescence spectroscopy, circular dichroism (CD), and agarose gel electrophoresis.

Results: The findings of UV-Vis and fluorescence spectroscopy showed that *A. sieberi* decreased DNA-AGE (Advanced glycation end products) formation. Based on the CD and agarose gel electrophoresis results, the structural changes of glycated DNA were decreased in the presence of *A. sieberi*.

Conclusion: Thus, *A. sieberi* has beneficial effects against DNA glycation and could be a promising agent for ameliorating the adverse effects of glycation in the presence of glucose and in conditions of raised blood glucose, such as diabetes, after confirmation in further studies.

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Introduction

Chronic hyperglycemia causes non-enzymatic DNA glycation, which is a series of cascade reactions between the amino groups of nucleic acids and the carbonyl groups of reducing sugars (1). The end products of this process are advanced glycation end products (AGEs), which are among the main known factors elevated in urine and tissue in animal models and contribute to the development of diabetes complications (2) as well as other diseases, including Parkinson's disease, Alzheimer's disease, and aging (3).

Herbal medicines with antiglycation and antioxidant activity have been crucial for preventing and alleviating AGE-mediated diabetes problems (4). Asteraceae (Compositae) is one of the largest and most widespread families of plants, with about 33,000 accepted species. *Artemisia* is a large, diverse genus of plants with more than 480 species belonging to Asteraceae (5), and these species have been studied in vitro and in vivo, as well as in clinical trials, for their anticancer, antimalarial, antibacterial, antiviral, and antidiabetic properties (6). For example, the essential oils of *Artemisia dracunculus* can be used as natural food preservatives due to their great antioxidant and antimicrobial properties (7).

Artemisia sieberi Besser is a shrubby aromatic plant distributed in Palestine, Syria, Iraq, Afghanistan, Pakistan, Central Asia, and Iran (8). It has a long history of use in traditional medicine. In traditional medicine, *A. sieberi* has been recommended for various illnesses and disorders, such as intestinal disturbances, coughing, inflammation, wound healing, and diabetes (9,10).

A. sieberi is a promising natural source that is rich in polyphenolic compounds such as flavones, apigenin, flavonoids, santonin, luteolin, sesquiterpene lactones, and bicyclic monoterpane glycosides; therefore, it has been suggested as a potential source of new antioxidant drugs (11-14). Furthermore, *A. sieberi* leaf extract possesses blood glucose-lowering action in diabetic conditions and could prevent diabetic complications associated with raised blood glucose (15,16).

Therefore, this study aimed to determine the antiglycation potential of *A. sieberi* extract in the presence of glucose using fluorescence, UV-Vis, and CD spectroscopy, as well as agarose gel electrophoresis.

Methods

Chemicals

We provided β -D-glucose, DNA from calf thymus, agarose, ethidium bromide, acetoacetate (AA), sodium dihydrogen orthophosphate, disodium hydrogen phosphate, EDTA, nitro-blue tetrazolium (NBT), sodium chloride, and Tris-HCl from Sigma-Aldrich (USA).

Preparation of AGE-DNA

DNA (25 μ g/mL) and D-glucose (130 mM) were mixed using a sodium phosphate buffer (200 mM; pH 7.4) in the presence or absence of *A. sieberi* (0.05%). After incubation for 4 weeks, the mixtures were dialyzed against sodium phosphate buffer for 48 h to remove unbound particles. The samples were then kept at -30 °C. The control was DNA incubated without glucose and the extract. The procedure for preparation of AGE-DNA was performed according to previous studies and our previously published studies (17-19).

Fluorescence analysis

Studies of fluorescence were carried out according to previously published procedures (18-20) using a spectrofluorophotometer (RF-5301-PC, Japan) at excitation wavelengths of 290 nm and 400 nm.

UV-Vis analysis

The UV-Vis analyses were performed using a Cary spectrophotometer (UV-2100, Rayleigh, China) according to previously published procedures (19,21). The absorbance of samples was recorded in a wavelength range of 200-600 nm.

Circular dichroism analysis

For CD studies, we used a spectropolarimeter (Jasco J-815, Japan) within the wavelength range of 220 - 400 nm. The procedure was in accordance with previous published studies (18,19).

Agarose gel electrophoresis

DNA agarose gel electrophoresis was performed for 2 h at 30 mA using 0.8% agarose gel. The buffer contained 40 mM Tris-acetate and 2 mM EDTA. After ethidium bromide staining, the bands were detected by UV (19,22).

Plant material and preparation of extract

Fresh leaves of the plant were collected in August 2023 from Zabol, Iran. The plant was botanically identified and authenticated in the Department of Biology, University of Zabol. Extraction was conducted based on the method described in a previous study (23). The leaves were shade dried at 30 - 35°C and the dried leaves were ground into coarse powder with an auto-mix blender. The powder obtained was macerated in 500 mL ethanol and water (50% V/V) at room temperature ($26 \pm 1^\circ\text{C}$) for 48 hours with occasional shaking. The filtrate was concentrated under reduced pressure at 50°C to give solid residues. The calculated yield ($21.54 \pm 0.03\%$) was kept in the dark at 4°C before the experiments.

Results

Fluorescence spectroscopy

The fluorescence emission spectra of all samples are shown in Figure 1. The DNA + Glc sample exhibited the highest emission intensity. The presence of *A. sieberi* significantly quenched this fluorescence, indicating a reduction in emission compared to the DNA + Glc group.

UV-visible spectroscopy

The UV-Vis absorption spectra are presented in Figure 2. Similar to the fluorescence results, the DNA + Glc sample showed the highest absorbance. The addition of *A. sieberi* reduced the absorbance by approximately 38%, demonstrating its interaction with the DNA complex.

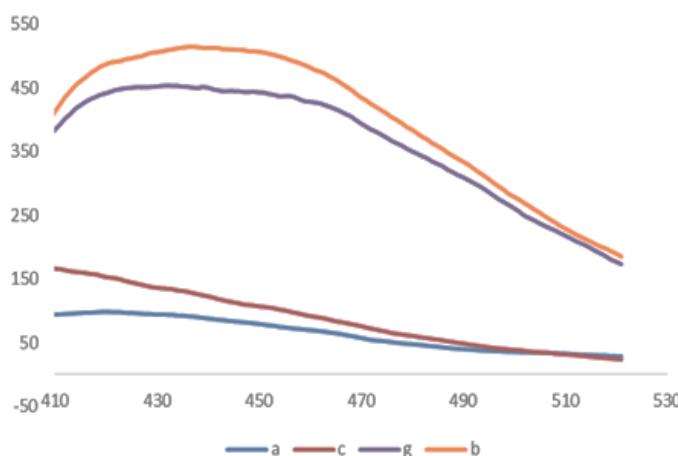


Figure 1. Fluorescence intensities of control-DNA (a), DNA + Sieberi (c), DNA + Glc + Sieberi (g), and DNA + Glc (b) after 4 weeks of incubation at 37°C in 200 mM phosphate buffer pH 7.4

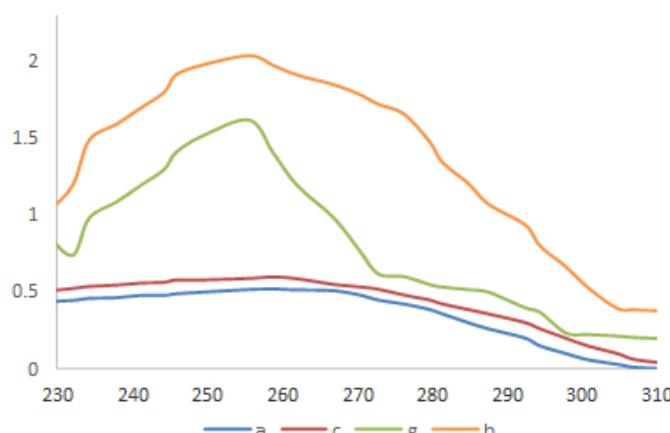


Figure 2. UV spectra of control-DNA (a), DNA + Sieberi (c), DNA + Glc + Sieberi (g), and DNA + Glc (b) after 4 weeks of incubation at 37°C in 200 mM phosphate buffer pH 7.4

CD analysis

Figure 3 shows the CD profile of all samples. The control DNA revealed a negative peak of -12 mdeg at 255 nm and a positive peak of +12 mdeg at 275 nm. Negative peaks of DNA + *A. sieberi*, DNA + Glc + *A. sieberi*, and DNA + Glc were -8.3, -3.4, and -2.2 mdeg at 245 nm, respectively. These samples also had positive peaks of 18.9, 13.1, and 10.4 nm, respectively.

Agarose gel electrophoresis

The electrophoresis analyses of all samples are depicted in Figure 4. The highest mobility was observed in DNA + Glc compared to the other groups. Incubation of *A. sieberi* with DNA and glucose dramatically decreased the mobility, as shown in the results of electrophoresis in Figure 4.

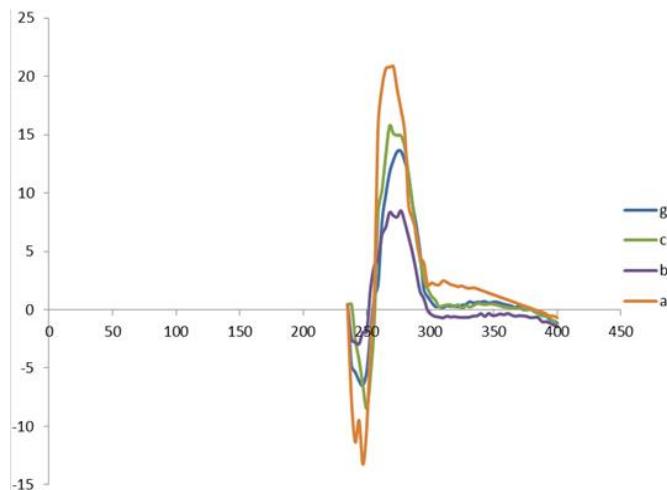


Figure 3. CD profile of control-DNA (a), DNA + Sieberi (c), DNA + Glc + Sieberi (g), and DNA + Glc (b) after 4 weeks of incubation at 37°C in 200 mM phosphate buffer pH 7.4

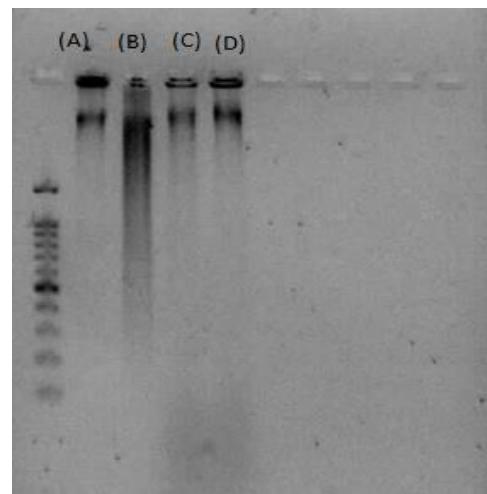


Figure 4. Agarose gel electrophoresis of native and modified DNA after 4 weeks of incubation at 37°C in 200 mM phosphate buffer pH 7.4: Lane (A), native DNA; lane (B), DNA + Glc; lane (C), DNA + *Artemisia*; lane (D), DNA + Glc + *Artemisia*.

Discussion

Although efforts to characterize structural and functional changes in proteins by glycation continue, detailed studies on non-enzymatic glycation of eukaryotic DNA have received minimal attention. It has been documented that accumulating AGEs on proteins and DNA contributes to the development of diabetes and age-related disorders (1,18). The DNA glycation process ultimately leads to DNA structural changes, strand breaks, and mutations (24). There are a number of compounds with inhibitory effects on glycation, such as vitamin B6 (25), aminoguanidine (26), quercetin (27), and aspirin (28). Investigations on glycation-inhibiting agents are important to identify their beneficial effects on preventing diabetes complications as well as some age-related neurodegenerative disorders.

Recently, herbal medicines with antiglycation and antioxidant activity have been mainly focused on for preventing and alleviating the problems related to AGEs accumulation (4). For example, *Nigella sativa* seed extract suppresses protein glycation in bovine serum albumin and also shows a strong capability for DNA damage protection (29). In the current study, *A. sieberi* extract could decrease the absorbance of DNA incubated with glucose according to the results of the UV–Vis analysis. According to a previous study, the UV-visible absorbance of glycated DNA increases because of the partial unfolding of the double helix and exposure of chromophoric bases (30). It has also been reported that glucose changes the biophysical and chemical characteristics of DNA (18,19). For example, glucose-treated DNA exhibits hyperchromicity, a decrease in melting temperature, and enhanced emission intensity in a time-dependent manner (24). This study was in vitro research that reports for the first time the effects of *A. sieberi* extract on the structural changes of glycated DNA. Therefore, based on the above explanations about the direct effects of glucose on DNA structure, it seems that *A. sieberi* likely reduces the UV-Vis absorbance of DNA through a combination of direct interactions with DNA and indirect effects mediated by its established antioxidant and ROS scavenging activities. Since ROS are potent mediators causing cellular stress originating from sugars auto-oxidation (31), the antioxidant activity of *A. sieberi* could be involved in the observed effects.

According to the findings of the fluorescence analysis, the emission of DNA + Glc + *A. sieberi* was decreased compared to the DNA + Glc sample. Based on previous studies, glycated DNA has an excitation of 400 nm and an emission of 290 nm (32). Therefore, it seems that the presence of *A. sieberi* has an inhibitory effect on DNA glycation and DNA structural changes by decreasing the fluorescence intensity. These results are consistent with one of our previous studies about the inhibitory effect of 3-b-hydroxybutyrate on decreasing the fluorescence intensity of DNA incubated with glucose (19).

The results of CD analysis revealed that the negative and positive parts of the CD spectra of DNA + Glc increased and decreased, respectively, compared to the CD spectra of control DNA. This was consistent with the findings of previous published studies (33). Furthermore, DNA showed fewer structural changes in the presence of glucose and *A. sieberi*. Therefore, incubation of this plant extract with DNA and glucose may produce fewer structural changes and, finally, DNA-AGEs formation. These findings are consistent with those of the UV–Visible results.

DNA incubated with glucose had higher mobility in electrophoresis compared to control DNA, which is in agreement with previous reports (28,29). However, DNA samples incubated with both glucose and *A. sieberi* had lower mobility according to the results of electrophoresis in this study. Therefore, the presence of *A. sieberi* has an inhibitory effect on further structural changes and damage of DNA compared to DNA + Glc.

Conclusion

The non-enzymatic glycation of eukaryotic DNA has been the subject of recent studies in the field of diabetes and its related complications. The present results are promising in showing protective properties of *A. sieberi* against DNA glycation and structural changes in the presence of glucose. As DNA glycation has an important role in the pathophysiology of diabetes, its complications, and also some neurodegenerative disorders like Parkinson's and Alzheimer's disease, this plant may be a potential source and candidate in the therapeutic field of these diseases after confirmation by further studies.

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Ethical statement

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

The study was planned by Musa Bohlooli. Farnush Sotudeh performed the experiments. Parisa Hasanein analyzed the data. The manuscript was written by Parisa Hasanein and Mohammad Hadadi. All authors have read and approved the manuscript for submission.

Data availability statement

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

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