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

Running title: Antidiabetic mechanism of Artemisia sieberi

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Abstract

Introduction: DNA Glycation damages DNA by inducing breaks of strands, mutations, and finally changes in gene expression, which is assumed as a main factor in the pathogenesis of diabetes and its complications. Therefore, antiglycation agents have been 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 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 were included UV–Vis, fluorescence spectroscopy, and circular dichroism (CD), and agarose gel electrophoresis.

Results: The findings of UV–Vis and fluorescence spectroscopy showed that *A. sieberi* decreased the 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 ameliorate the adverse effects of glycation in the presence of glucose and conditions of raised blood glucose like diabetes after confirming in further studies.

Keywords: Glycation, *Artemisia sieberi*, AGE, DNA, Glucose

Introduction

Chronic hyperglycemia causes non-enzymatic DNA glycation, which is a series of cascade reactions between the amino groups of nucleic acids and carbonyl groups of reducing sugars (1). The end products of this process are advanced glycation end products (AGEs) which are among the main know are elevated in urine and tissue in an animal model cause in producing diabetes complication (2) as well as other diseases including Parkinson, Alzheimer's 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 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) which 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 deracunculus* can be used as natural food preservatives due to great antioxidant and antimicrobial properties (7). *A. 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 monoterpene glycosides, therefore it has been suggested a as a potential source of new antioxidant drugs (11-14).

Furthermore, *A. sieberi* leaves 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. siebra* extract in the presence of glucose using fluorescence, UV–vis, and CD spectroscopy and 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 µg/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 by sodium phosphate buffer for 48 h to remove unbound particles. The samples were then kept at - 30 ºC. The control was DNA incubated without glucose and the extract. The procedure of preparation of *AGE-DNA* was performed according to the the previous studies and our previous published studies (17-19).

Fluorescence analysis

Studies of fluorescence were done according the previous published procesures (18-20) using a spectrofluorophotometer (RF-5301-PC, Japan) at excitation wavelength of 290 and 400 nm.

UV-vis analysis

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

Circular dichroism (CD) analysis

For carrying on CD studies, we used a spectropolarimeter (Jasco J-815, Japan) within the wavelenght of 220–400 nm. The procedure was according the previous published studies (18,19).

Agarose gel electrophoresis

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

Plant material and preparation of extract

Fresh leaves of the plant were collected in the month of 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 by previous study (23). The leaves were shade dried at (30–35) °C and the dried leaves were ground into coarse powder with auto-mix blender. The powder obtained was macerated in 500 ml ethanol and water (50% V/V) at room temperature (26 ± 1°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 ± 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.

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 pick 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 pick 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 related to DNA + Glc compared to other groups. Incubation of *A. sieberi* with DNA and glucose has dramatically decreased the mobility as shown in the results of electrophoresis in Figure 4.

Discussion

Although efforts to characterize structural and functional changes in proteins by glycation continue, fine studies on nonenzymatic glycation of eukaryotic DNA have received minimal attention. It has been documented that accumulating AGEs on proteins and DNA contributes to developing diabetes and age-related disorders (1, 18). DNA glycation process finally leads to DNA structural changes, strand breaks, and mutations (24). There are a number of compounds with inhibitory effects on glycation, such as vitamin B_6 (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 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 showed a strong capability for DNA damage protection (29). In the current study, *A. sieberi* extract could decrease the absorbance of DNA incubated with glucose compared to according to results of the UV–Vis. According to a previous study, UV-visible absorbance of glycated DNA increases because of the partial unfolding of double helix and exposure of chromophoric bases (30). It has been also reported that glucose makes changes in biophysical and chemical characterization of DNA (18, 19). For example, glucose treated DNA exhibits hyperchromicity, 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, according to 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 activity and ROS scavenging activity. Because ROS are a potent mediator 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 the previous studies, the 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 CD spectra of the DNA + Glc increased and decreased respectively compared to CD spectra of control-DNA. This was consistent with the findings of previous published studies (33). Furthermore, DNA showed less 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 that 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, in DNA samples incubated with both glucose and *A. sieberi* had lower mobility according to the results of electrophoresis in this study. Therefore, presence of *A. sieberi* has an inhibitory effect on more structural changes and damage of DNA compared to DNA+Glc.

Conclusions

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 pathophysiology of diabetes, its complications and also some neurodegenerative disorders like Parkinson and alzheimer's disease, this plant may be a potential source and candidate in the therapeutic field of these diseases after confirming by further studies.

Conflict of interests

The authors state no conflict of interest.

Author contributions

The study was planned by Musa Bohlooli. Farnush Sotudeh did 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 submitting.

Data availability statement

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

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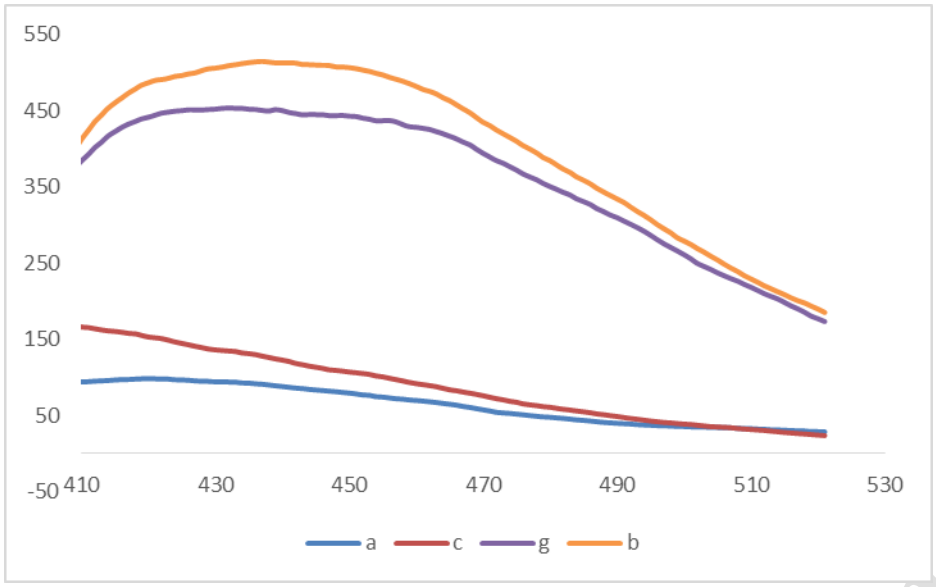

Figure 1. Fluorescence intensities of control-DNA (a), DNA + *Sieberi* (c), DNA + Glc + *Sieberi* (g) and DNA + Glc (b) after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4.

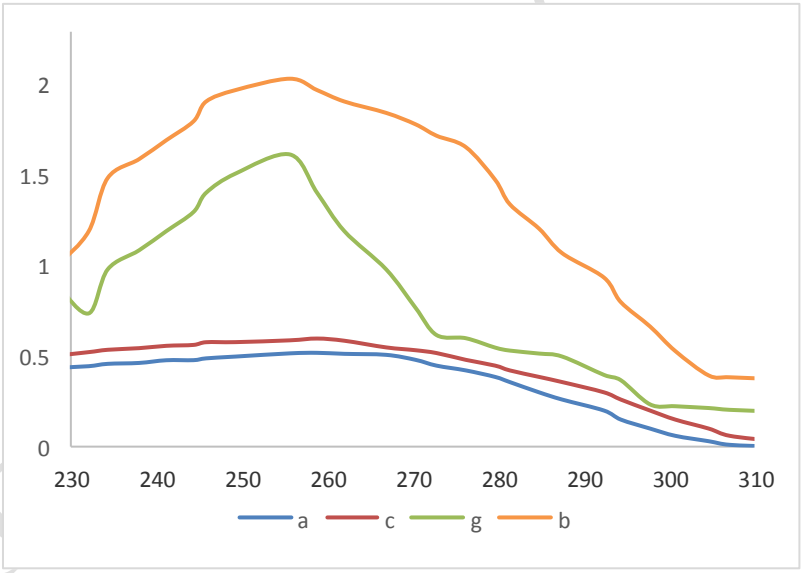

Figure 2. UV spectra of control-DNA (a), DNA + *Sieberi* (c), DNA + Glc + *Sieberi* (g) and DNA + Glc (b) after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4.

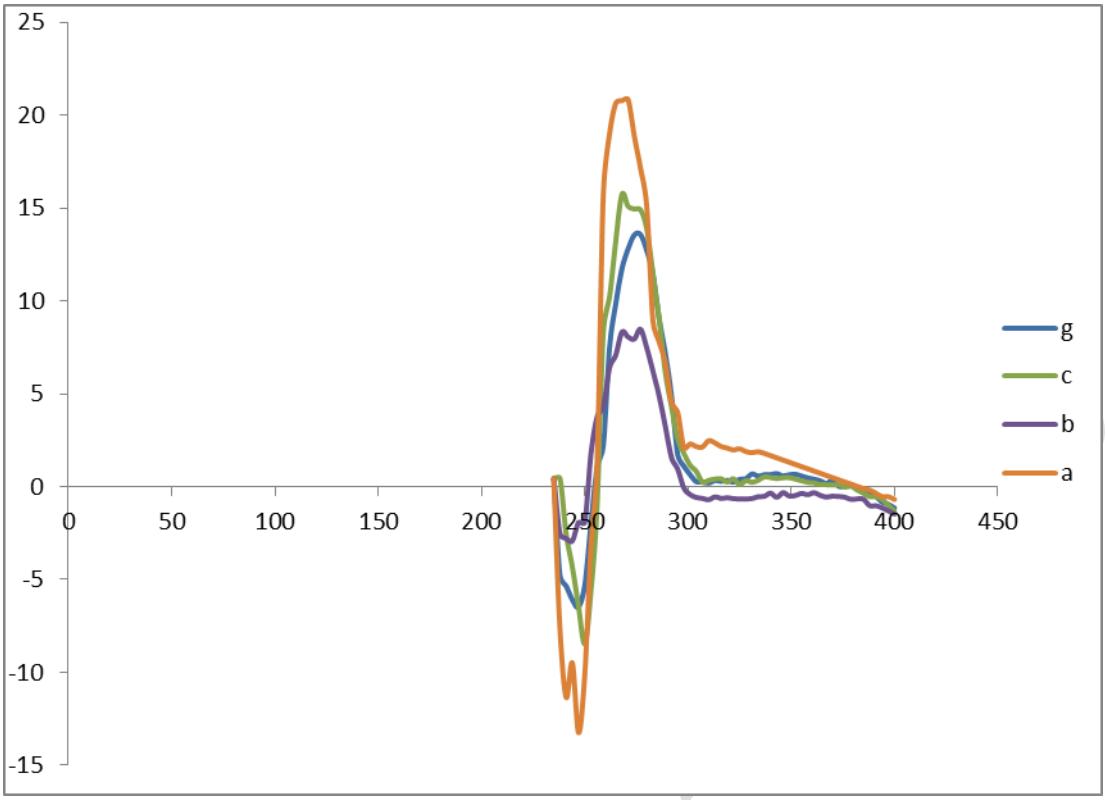

Figure 3. CD profile of control-DNA (a), DNA + *Sieberi* (c), DNA + Glc + *Sieberi* (g) and DNA + Glc (b) after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4.

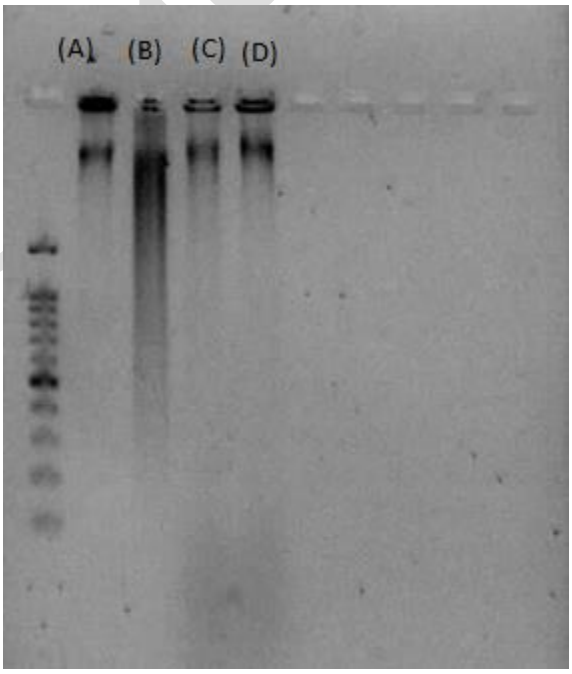

Figure 4. Agarose gel electrophoresis of native and modified DNA after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4: Lane (A), native DNA; Lane (B), DNA + Glc; Lane (C), DNA + *Artemisia*; Lane (D), DNA + Glc + *Artemisia*.