Attenuation of glucose mediated DNA glycation by *Tamarix aphylla* extract

Running title: Inhibitory eefcts of Tamarix aphylla on DNA glycation

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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 pathogenesis of diabetes and its complications. Therefore, antiglycation agents have been focused recently for preventing and alleviating diabetes complications. According to the reported antidiabetic effects of *Tamarix aphylla* (*T. aphylla*) leaves extract, this study was aimed to determine the effect of *T. aphylla* on glucose-mediated DNA glycation for the first time.

Methods: DNA incubated with glucose for 4 weeks and the inhibitory or fascilitatory effects of *T. aphylla* on DNA structural changes were studied by various techniques. These techniques were included UV–Vis, fluorescence spectroscopy, circular dichroism (CD) and agarose gel electrophoresis.

Results: The findings of UV–Vis and fluorescence spectroscopy showed that *T. aphylla* 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 *T. aphylla*.

Conclusion: Thus *T. aphylla* 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 after confirming in further studies.

Keywords: Glycation, *Tamarix aphylla*, 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 n 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). *Tamarix aphylla* (*T. aphylla*), a medium sized tree widely distributed in the Middle East and parts of Southern and Western Asia (5). It has a long history of use in traditional medicine. In traditional medicine, *T. aphylla* has been recommended for various illnesses and disorders, such as antipyretic, antimicrobial, antifungal, analgesic, antirheumatic, and anti-inflammatory (6-8).

T. aphylla is a promising natural source that is rich in polyphenolic compounds such as flavonoids, phenolic acids, tannins and coumarins, therefore it has been suggested a as a potential source of new antioxidant drugs (8-10). Furthermore, *T. aphylla* leaves extract possesses blood glucose lowering action in diabetic condition and could prevent diabetic complication associated with raise blood glucose (11). Therefore, this study was aimed to determine the antiglycation potential of *T. aphylla* extract in the presence of glucose using UV-vis, fluorescence 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 *T. aphilla* (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 (12-14).

UV-vis analysis

The UV–Vis analyses were done via a Cary spectrophotometer (UV-2100, Rayleigh, China) according to the previos published procedures (14,15). The absorbance of samples was recorded in a wavelength range of 200-600 nm.

Fluorescence analysis

Studies of fluorescence were done according the previous published procesures (13,14,16) using a spectrofluorophotometer (RF-5301-PC, Japan) at excitation wavelength of 290 and 400 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 (13,14,17).

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 (14,18).

Plant material and preparation of extract

Fresh leaves were collected from the tree of *T. aphylla* L. in the month of April 2021 from Zabol, Iran. The plant was botanically identified and authenticated by Dr. Esmaelzadeh in the Department of Biology, University of Zabol. Extraction was conducted based on the method described by previous studies (19). 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 (3.24 kg) 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 40 °C until extraction solvent was removed. A dark green soluble crude residue was obtained (about 214.27 g, 6.61% w/w).

Results

UV-visible spectroscopy

Figure 1 demonstrates the UV-Vis spectra of all samples including control-DNA, DNA + *T. aphilla*, DNA + Glc + *T. aphylla* and DNA + Glc. These results indicated that the highest absorbance is related to DNA + Glc. Furthermore, presence of *T. aphylla* in this study has decreased absorbance by approximately 42.85% as shown in Figure 1.

CD analysis

Figure 2 shows the CD profile of all samples. The control-DNA revealed a negative peak of -8 mdeg at 255 nm, and a positive peak of +17 mdeg at 275 nm. Negative pick of DNA + *T. aphylla*, DNA + Glc + *T. aphylla* and DNA + Glc were -6.3, -4.4 and -3.2 mdeg at 245 nm, respectively. These samples had also positive pick of 16.9, 12.1 and 11.9 nm, respectively.

Fluorescence spectroscopy

The fluorescence spectra result for all samples were depicted in Figure 3. As shown in Figure 3, the highest intensity of the fluorescence emission was related to DNA + Glc. These results revealed that *T. aphylla* could decrease the fluorescence emission of DNA compared to DNA +Glc group.

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 *T. aphylla* 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 glycationof 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, 13). DNA glycation process finally leads to DNA structural changes, strand breaks, and mutations (20). There are a number of compounds with inhibitory effects on glycation, such as vitamin B_6 (21), aminoguanidine (22), quercetin (23) and aspirin (24). 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 suppress protein glycation in bovine serum albumin and also showed a strong capability for DNA damage protection (25). In the current study, *T. aphylla* 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 (26). It has been also reported that glucose makes changes in biophysical and chemical characterization of DNA (13, 14). For example, glucose treated DNA exhibits hyperchromicity, decrease in melting temperature, and enhanced emission intensity in a time dependent manner (20). This study was an *in vitro* research that reports for the first time the effects of a *T. aphylla* extract on the structural changes of glycated DNA. Therefor according to the above explanations about the direct effects of glucose on DNA structure, it seems that *T. aphylla* 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 (27), the antioxidant activity of *T. aphylla* could be involved in the observed effects.

According to the findings of the fluorescence analysis the emission of DNA + Glc + *T. aphylla* 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 [28]. Therefore, it seems that the presence of *T. aphylla* 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 (29).

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 (30). Furthermore, DNA showed less structural changes in the presence of glucose and *T. aphyla*. Therefore, incubation of this plant extract with DNA and glucose may produce less 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 *T. aphylla* had lower mobility according to the results of electrophoresis in this study. Therefore, presence of *T. aphylla* has an inhibitory effect on more structural changes and damage of DNA compared to DNA+Glc.

Conclusion

Although efforts to characterize structural and functional changes in proteins by glycation continue, fine studies on nonenzymatic glycationof eukaryotic DNA have received minimal attention. This study provides promising and scientific findings on the beneficial effects of *T. aphylla* on DNA glycation and structural changes in the presence of glucose. *T. aphylla* had an inhibitory effect on DNA structural changes and finally AGEs formation. Decreased

AGEs formation was showed by UV-vis and fluorescence spectrometry. Incubation of this plant extract with DNA and glucose decreased the UV-vis absorbance as well as the fluorescence intensity by approximately 33.6% and 26.6%, respectively. It seems that these effects may be mediated by direct interactions with DNA and indirect effects originated from established antioxidant activity of *T. aphylla* and ROS scavenging activity. Furthermore, the observed effects might be related to its preserving effect on the global fold of the DNA molecule that means stacking with glycogenic nucleotides which direct the ligands to the glycogenic core, thereby counteracting the effect of the formed inter-strand cross-link in the duplex DNA. Whatever the mechanisms would be, the results of the present study deserve further studies to obtain a better understanding of the detailed mechanisms.

Conflict of interests

The authors state no conflict of interest.

Authors' contributions

The study was planned by Musa Bohlooli. Fahime Javadi Hedaiat Abad and Neda Poormoalei did all the experiments. Parisa Hasanein and Mostafa Khajeh analyzed the data. The manuscript was written by Parisa Hasanein, and Neda Poormolaei. Plant material authentication was carried out by Sedigheh Esmaielzadeh Bahabadi. All authors have read and approved the manuscript for submitting.

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Ethical Statement

Since this work was an in vitro study and we analysed the DNA of a plant with several biophysical methods, therefore it needs no ethical statement.

References

1. Voziyan PA., Khalifah RG, Thibaudeau C, et al. Modification of proteins in vitro by physiological levels of glucose: pyridoxamine inhibits conversion of Amadori intermediate to advanced glycation end-products through binding of redox metal ions. J Biol Chem. 2003; 27; 46616–46624.
2. Stitt AW. Advanced glycation: an important pathological event in diabetic and age-related ocular disease. Br J Ophthalmol. 2001; 85: 746–753.
3. Munch G, Shepherd CE, McCann H, et al. Intraneuronal advanced glycation end products in presenilin-l Alzheimer's disease. Neuroreport 2002; 13: 601–604.
4. Thilavech T, Marnpae M, Mäkynen K, Adisakwattana S. Phytochemical composition, antiglycation, antioxidant activity and methylglyoxal-trapping action of brassica vegetables. Plant Foods Hum Nutr. 2021; 76; 340–346.
5. Thilavech, T.; Marnpae, M.; Mäkynen, K.; Adisakwattana, S. Phytochemical Composition, Antiglycation, Antioxidant Activityand Methylglyoxal-Trapping Action of Brassica Vegetables. Plant Foods Hum. Nutr. 2021, 76, 340–346.].

6. Qadir, M.I.; Abbas, K.; Hamayun, R.; Ali, M. Analgesic, anti-inflammatory and anti-pyretic activities of aqueous ethanolic extractof Tamarix aphylla L. (Saltcedar) in mice. Pak. J. Pharm. Sci. 2014, 27, 1985–1988.
7. Alshehri SA, Wahab S, Abullais SS, et al. Pharmacological efficacy of *Tamarix aphylla*: A Comprehensive Review. Plants (Basel) 2022 Jan; 11(1): 118;
8. Gadallah AS, Atta-ur-Rahman M-R, Yousuf S M, et al. Anti-Inflammatory principles from *Tamarix aphylla* L.: A bioassay-guided fractionation study. Molecules 2020 Jul; 25(13): 2994.
9. Mahfoudhi A, Salem AB, Garrab M, et al. Antioxidant and antimicrobial activities of Tamarix aphylla (L.) Karst. growing in Tunisia. Mor J Chem. 2016, 4(4): 987-995.
10. Qnais E, Alqudah A, Wedyan M, et al. Potential anti-inflammatory activity of the Tamarix aphylla essential. Pharmacia 2023; 70 (3): 707-771.
11. Ahmed Tariq RU, Khan N, Sharif N, Din ZU, Mansoor K. Antihyperglycemic effect of methanol extract of *Tamarix aphylla* L. Karst (Saltcedar) in streptozocin-nicotinamide induced diabetic rats. Asian Pacific J Tropic Biomed. 2017; 7 (6): 619-623.
12. Ashraf JM, Arif B, Dixit K, Alam K. Physicochemical analysis of structural changes in DNA modified with glucose. Int J Biol Macromol. 2012; 51: 604–611.
13. Bagherzadeh-Yazdi M, Bohlooli M, Khajeh M, Ghamari F, et al. Acetoacetate enhancement of glucose mediated DNA glycation. Biochem and Biophysic Reports 2021; 25:100878.
14. Bohlooli M, Miri M, Khajeh M, et al Inhibitory influence of 3-β-hydroxybutyrate on calf thymus DNA glycation by glucose. RSC Advances 2016; 87: 83880-83884.
15. Chobot A, Górowska-Kowolik K, Sokołowska M, Jarosz-Chobot, P. Obesity and diabetes—not only a simple link between two epidemics. Diabetes/Metabolism Res. Rev. 2018, 34, e3042.
16. Ali A, More TA, Hoonjan AK, Sivakami S. Antiglycating Potential of Acesulfame Potassium: An Artificial Sweetener. Appl Physiol Nutr Metab 2017; 42; 1054–1063.
17. Bagherzadeh-Yazdi M, Bohlooli M, Khajeh M, Ghamari F, et al. Acetoacetate enhancement of glucose mediated DNA glycation. Biochem and Biophysic Reports 2021; 25:100878.
18. Mustafa I, Ahmad S, Dixit K, Moinuddin, Ahmad J, Ali A. Glycated human DNA is a preferred antigen for anti-DNA antibodies in diabetic patients. Diabetes Res Clin Pract. 2012; 95: 98–104.
19. Al-Otibi F, Moria GA, Alharbi RI, Yassin MT, Abdulaziz A, Al-Askar A. The antifungal properties of tamarix aphylla extract against some plant pathogenic fungi. Microorganisms. 2023; 11(1): 127.
20. Ashraf JM, Arif B, Dixit K, Moinuddin M, Alam K. Physicochemical analysis of structural changes in DNA modified with glucose. Int J Biol Macromol. 2012; 51: 604–611.
21. Booth AA, Khalifah RG, Hudson BG. Thiamine pyrophosphate and pyridoxamine inhibit the formation of antigenic advanced glycation end-products: comparison with aminoguanidine, Biochem Biophys Res Commun. 1996; 220: 113–119.
22. Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 1986; 232: 1629–1632.
23. Morimitsu Y, Yoshida K, Esaki S, Hirota A. Protein glycation inhibitors from thyme (Thymus vulgaris). Biosci Biotechnol Biochem 1995; 59: 2018–2021.
24. Urios P, Grigorova-Borsos AM, Sternberg M. Aspirin inhibits the formation of pentosidine, a cross-linking advanced glycation end product, in collagen, Diabetes Res Clin Pract. 2007; 77: 337–340.

25. Balyan P, Shamsul Ola M, Alhomida AS, Ali A. D-ribose-induced glycation and its attenuation by the aqueous extract of nigella sativa seeds. Medicina (Kaunas) 2022; 58(12): 1816.
26. Ahmad S, Dixit K, Shahab U, Alam K and Ali A. Genotoxicity and immunogenicity of DNA-advanced glycation end products formed by methylglyoxal and lysine in presence of Cu^2. Biochem. Biophys. Res. Commun. 2011, 407, 568–574.
27. Thornalley, PJ. Monosaccharide autoxidation in health and disease. Environ. Health Perspect. 1985*;* 64:297–307.
28. Ahmad S, Shahab U, Baig MH, et al. Inhibitory effect of metformin and pyridoxamine in the formation of early, intermediate and advanced glycation end-products. PloS One 2013; 8; e72128.
29. Bohlooli M, Miri M, Khajeh M, et al. Inhibitory influence of 3-β-hydroxybutyrate on calf thymus DNA glycation by glucose. RSC Advances 2016; 87: 83880-83884.
30. Ahmad S, Moinuddin M, Shahab U, et al. Glycoxidative damage to human DNA: neo-antigenic epitopes on DNA molecule could be a possible reason for autoimmune response in type 1 diabetes. Glycobiol. 2014; 24; 281–291.

Figures

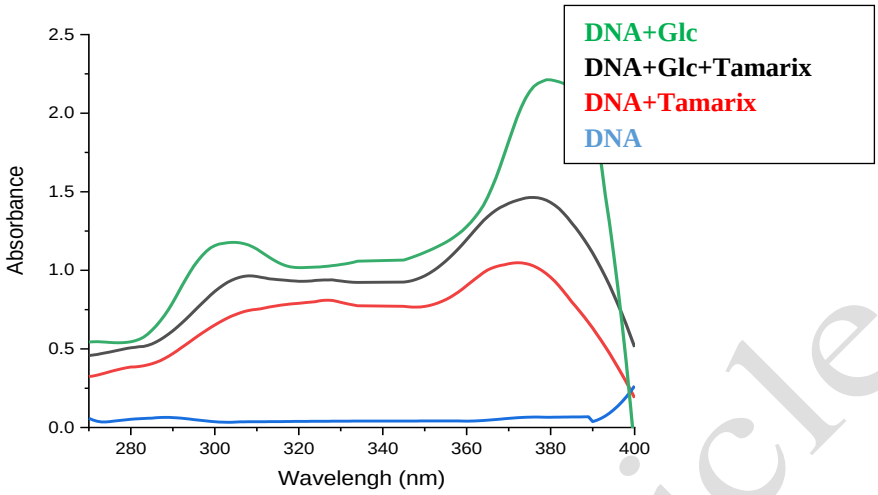

Figure 1. UV spectra of control-DNA, DNA + *Tamarix*, DNA + Glc + *Tamarix* and DNA + Glc after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4.

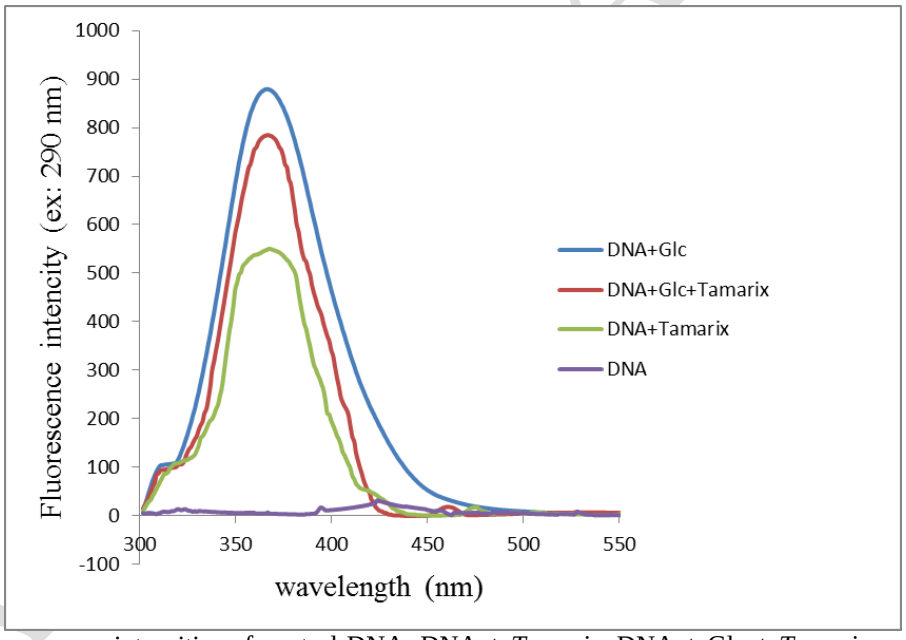

Figure 2. Fluorescence intensities of control-DNA, DNA + *Tamarix*, DNA + Glc + *Tamarix* and DNA + Glc after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4.

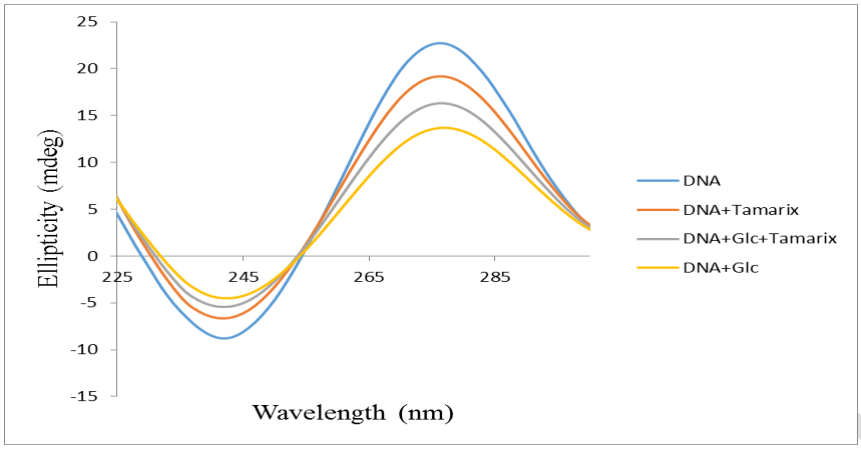

Figure 3. CD profile of control-DNA, DNA + *Tamarix*, DNA + Glc + *Tamarix* and DNA + Glc after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4.

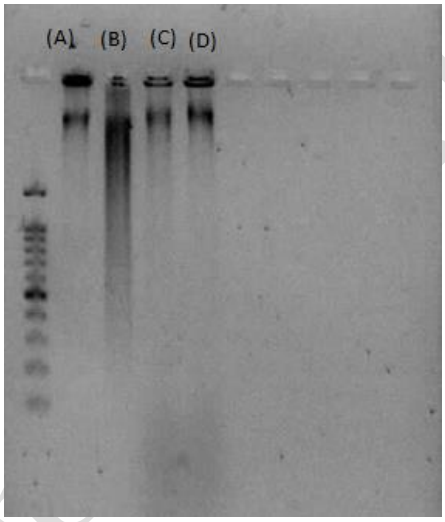

Figure. 4. Agarose gel electrophoresis of native and modified DNA after 4 weeks of incubation at 37 ºC in 200 mM phosphate buffer pH 7.4: Lane (A), native DNA; Lane (B), DNA + Glc; Lane (C), DNA + *Tamarix*; Lane (D), DNA + Glc + *Tamarix*.