Current Perspectives on
Medicinal & Aromatic Plants

Nigella sativa Seed Extracts Prevent the Glycation of Protein and DNA

Rashmi Pandey, Dinesh Kumar, Ahmad Ali*

Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai, Maharashtra, India

*Correspondence: ahmadali@mu.ac.in (Ahmad Ali), Tel: +919870941656

Received: 19 May 2018; Accepted: 08 July 2018; Published: 28 July 2018

Abstract

Objectives: Diabetes has become a major health and socioeconomic issue for India in last one decade. The condition is identified by an increased blood level which leads to generation of many harmful products collectively known as advanced glycation end products (AGEs). These products have been implicated in the secondary complications of Diabetes. Both artificial and natural compounds have been used to prevent the accumulation of AGEs. The present study was designed to investigate the role of *Nigella sativa* seeds in the inhibition of early and advanced glycation products *in vitro*. *Methods:* BSA was glycated in the presence of fructose for 28 days at 37 ºC in the presence and absence of seed extracts. The amount of glycation products was measured by established methods like browning, NBT assay, and DNPH methods. The effect of black cumin seeds was also checked on glycation of DNA and the sample was analyzed by agarose gel electrophoresis. *Results:* In the presence of black cumin seed extracts, there was a significant decrease in the amount of early and advanced glycation products as compared to BSA + fructose sample. The seed extracts also reversed the glycation-induced DNA damage. *Conclusion:* The results indicate the inhibitory role of Nigella sativa in the process of glycation of proteins and DNA.

Keywords: Advanced glycation end-products (AGEs), Antiglycation, Browning, DNA damage, *Nigella sativa* (Black cumin)

1. Introduction

The glycation reaction was first stated by French chemist Louise Camille Millard in 1912, Hence this reaction is also named as Millard reaction (Kikuchi et al., 2003). Glycation is the result of covalent bonding of a free amino group of biomolecules with a reducing sugar (Ahmed, 2005). This results in the formation of a Schiff base that undergoes rearrangement, dehydration and cyclization to form a more stable Amadori product. The final products of non-enzymatic glycation of biomolecules like DNA, proteins and lipids are known as advanced glycation end products (AGEs). These products are generated and accumulate in the body depending on the time and concentration of glucose (Brownlee et al., 1984). Some of these AGEs have been characterized and implicated in various pathophysiological disorders. Although the exact mechanism by which AGEs cause diseases is not understood fully but they have been shown to alter the structure of proteins and other biomolecules. Another mechanism by which glycation products cause deleterious effects is through the generation of free radicals (Ahmad et al., 2014).

Glycation has both physiological and pathophysiological significances. Under physiological conditions, glycation can be detected in the ageing process, and the

reactions are more rapid and intensive with frequent increase of glucose concentration. Acute and chronic hyperglycemia is known to enhance early, intermediate and advanced glycation (Jakus et al., 2004). In recent years, researchers are focusing more on medicinal plants and their extracts, which could be used as alternatives to synthetic compounds; to improve metabolism and to ameliorate the conditions in many diseases. Many studies have been carried out to explore the probable beneficial effects of herbal plants with medicinal properties such as curcumin, rutin, garcinol and flavonoid-rich extracts (Khan and Gothalwal, 2018); these are known to possess antioxidative and antiglycating properties that prevent glycation process *in vitro* and *in vivo*. *Nigella sativa* (Black cumin seeds) is considered one of such plants having pharmacological properties, including antioxidant, anti-inflammatory, hypoglycaemic and anti-hypertensive properties (Najmi et al., 2012). In the present study methanolic extract of Black cumin seed was used to investigate its effect on glycated proteins and DNA. It was found that the extracted seeds prevented the accumulation of glycation products and glycation-induced DNA damage. Further studies can be designed to identify the bioactive compound from the *Nigella sativa* seeds and also the stage at which the glycation is prevented.

2. Materials and Methods

The Black cumin seeds were purchased from local market of Mumbai and the following chemicals were purchased as indicated: Agarose, methyl glyoxal and Bovine serum albumin were purchased from Sigma-Aldrich. Lysine was procured from Himedia. pBR322 was purchased from Thermo Fisher. All other chemicals used were of high analytical grade.

Extraction of Black cumin: The Black cumin seeds were extracted by hot extraction method. K1 - methanol was used as solvent. 15 g of seeds were soaked in 60 mL of methanol, boiled gradually and filtered at RT and in a weighed beaker kept for drying in thermostat at 50°C. The residue (90 mg) obtained was dissolved in 10 mL methanol (9 mg/mL). K2 - water was used as solvent. 15 g of seeds were soaked in 60 mL of methanol, boiled gradually and filtered at RT and in a weighed beaker kept for drying in thermostat at 50°C Residue (363 mg) obtained was dissolved in 20 ml methanol (18.15 mg/mL).

Incubation of Nigella sativa seeds extracts with in vitro glycation system: An aqueous solution of BSA (10 mg/mL) was incubated with fructose (100 mg/mL) and with or without seed extracts (1 mg/mL) in 0.1 M phosphate buffer (pH 7.4) at 37 °C for 28 days. Bacterial contamination was prevented by addition of 3 mmol/L sodium azide.

Measurement of browning: The extent of browning was measured at 420 nm, using Shimadzu UV 1800 spectrophotometer (Ali et al., 2017) and relative percentage of absorbance was used to plot the graph.

Measurement of Fructosamines: The concentration of fructosamine, an Amadori product, was measured by Nitroblue-tetrazolium (NBT) assay as described by Banan and Ali (2016) with minor modifications. 10 µL of the glycated sample was incubated with 100 µL of 0.5 mM NBT in 0.1 M carbonate buffer (pH 10.4) at 37 °C for 15 minutes. The volume of the reaction mixture was made to 1.0 mL with distilled water and the absorbance was measured at 530 nm using Shimadzu UV 1800 spectrophotometer.

Determination of Protein carbonyl content: The carbonyl group in glycated sample was determined by the conventional DNPH method as s described previously (Meeprom et al.,

2013; Banan and Ali, 2016) with minor modifications. In this method, 400 µL of 10 mM DNPH in 2.5 M HCl was added to 100 µL of glycated samples and incubated in dark for an hour. Then, 500 µL of 20% (w/v) TCA was added and kept on ice for 5 minutes for proteins to precipitate. The tubes were centrifuged at 10,000 rpm for 10 min at 4 °C, following which the protein pellet was washed with 500 µL of ethanol/ethyl acetate (1:1) mixture three times. The pellet was resuspended in 100 µL of 6 M guanidine hydrochloride and distilled water was added to make the volume sufficient for spectroscopic measurement (1.0 mL) at 370 nm using Shimadzu UV 1800 spectrophotometer.

Determination of protein aggregation index: The effect of black cumin seed extracts was also checked on the protein aggregation by measuring the absorbance of glycated samples in the presence and absence of seed extracts. The aggregation index was calculated by the following formula

Percentage of protein aggregation index = A340 / (A280 – A340) * 100

In vitro glycation of plasmid DNA in the presence of Black cumin seed extract: The effect of black cumin seed extract on the glycation-mediated DNA strand breakage was performed according to a previous publication with minor modifications (Ali et al. 2014). pBR322 plasmid DNA (0.25 µg) in 100 mM potassium phosphate buffer (pH 7.4) was incubated with lysine (20 mM), MG (20 mM) and $FeCl_3$ (100 µM) in presence and absence of black cumin seed extract (K1 and K2 – 10 µg/ml)). The reaction mixture of samples was incubated at 37°C for two hours. pBR322 plasmid DNA (0.25 µg) in 100 mM potassium phosphate buffer (pH 7.4) without glycation system and black cumin seed extract was used as control. The reaction was stopped by freezing the samples at −20 °C.

Agarose gel electrophoresis of glycated plasmid DNA sample: Ten microliters of samples were mixed with 2 µL of 6X gel loading dye and loaded on to 1% agarose gel. Electrophoresis was carried out initially at 90 V and once the samples left the well, voltage was decreased to 85 V. As soon as the dye band reached two-thirds of gel length, electrophoresis was terminated and gel was stained using ethidium bromide solution (final concentration 5 µg/mL) for 20 min in dark. Subsequently the gel was visualized under Gel-Doc and bands analyzed with the help of control.

3. Results and Discussion

Effect of Nigella sativa seed extracts on Browning: BSA was glycated with fructose at 37°C for 28 days in the presence and absence of black cumin seed extracts. The extent of browning, an indicator of glycation, was measured at 420 nm. It can be seen from the graph (Fig. 1) that both the extracts caused significant decrease in the browning of BSA by fructose. However, K2 caused more inhibition (47.96 %) of glycation as compared to K1 (34.94 %).

Effect of Nigella sativa seed extracts on Fructosamine: NBT assay is a reliable method for the meaurement of fructosamines, the early glycation products. When the amount of fructosamine was compared between the glycated sample in the presence and absence of black cumin seed extracts it was found that K2 (39.42 %) was more potent in preventing the formation of fructosamines as compared to K1 (14.59 %) with respect to control (BSA + fructose) (Fig. 2).

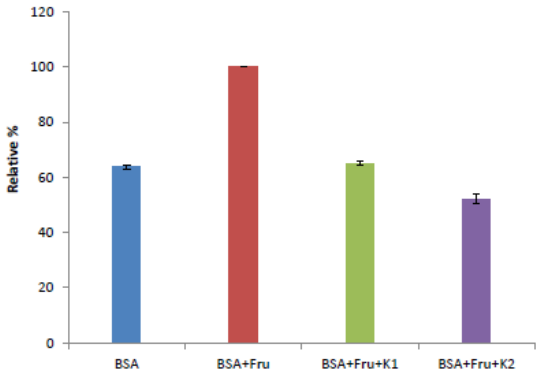


Fig.1: Measurement of Browning
BSA and fructose were incubated with black cumin seed extracts (K1 and K2) at 37 °C for 28 days. The absorbance was taken at 420 nm and tube containing BSA + fructose was used as standard (100%). BSA: Bovine Serum Albumin, Fru: Fructose, K1 and K2 – black cumin seed extracts.

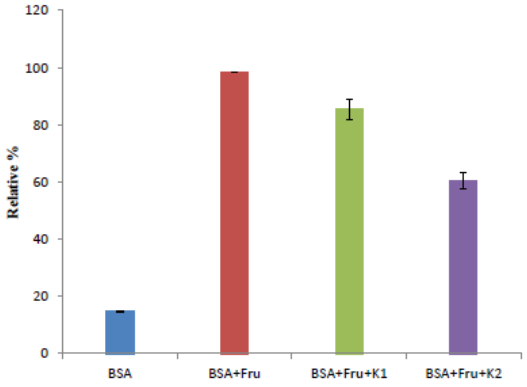


Fig.2: Measurement of Fructosamine
The amount of fructosamines was measured in the glycated sample in the presence or absence of K1 and K2. Absorbance was taken at 530 nm and relative absorbance was plotted on the graph using BSA + fructose tube as the standard (100%). BSA: Bovine Serum Albumin, Fru: Fructose, K1 and K2 – black cumin seed extracts.

Effect of Nigella sativa seed extracts on the Carbonyl content: The amount of carbonyl content was measured in the glycated sample in the presence or absence of black cumin extracts by DNPH method. Absorbance was taken at 370 nm and relative absorbance was plotted on the graph using BSA+Fructose as the standard (100%). It was observed that carbonyl content was significantly reduced by 58.28% in K1 and 57.06% in K2 as compared to glycated BSA (Fig. 3).

Effect of Nigella sativa seed extracts on Protein Aggregation Index: Aggregation of protein is the late stage of non- enzymatic glycation process in which there is formation of cluster when the carbonyl group bound to biomolecules of protein. The aggregation index showed very significant reduction of amyloid cross-β structure in presence of K1 and K2 in comparison Fructose glycated protein (Fig. 4).

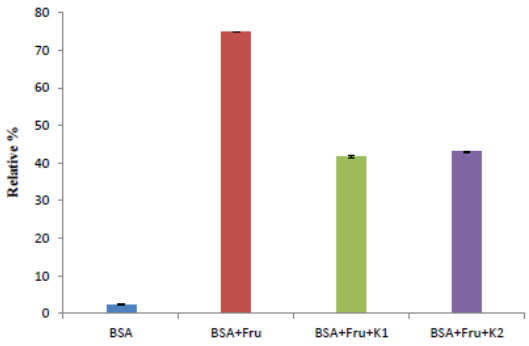


Fig.3: Measurement of Carbonyl Content
The amount of carbonyl content was measured in the glycated sample in the presence or absence of K1 and K2. Absorbance was taken at 370 nm and relative absorbance was plotted on the graph using BSA + fructose tube as the standard (100%). BSA: Bovine Serum Albumin, Fru: Fructose, K1 and K2 – black cumin seed extracts.

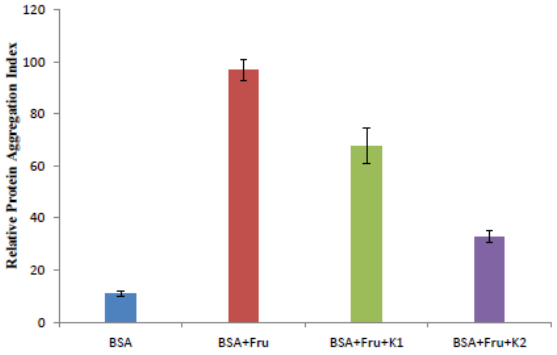
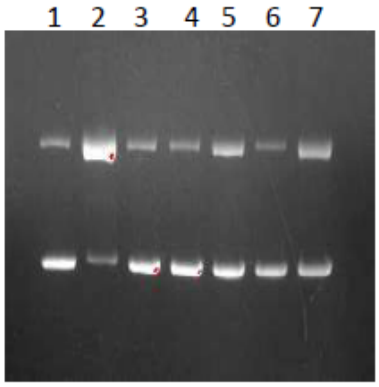


Fig.4: Measurement of Protein Aggregation Index
The absorbance of the glycated sample in the presence and absence was measured at 280 nm and 340 nm. The protein aggregation index was calculated by using the formula mentioned in the methods. Relative aggregation index was plotted on the graph using BSA + fructose tube as the standard (100%). BSA: Bovine Serum Albumin, Fru: Fructose, K1 and K2 – black cumin seed extracts.

Effect of Nigella sativa seed extracts on the glycation of DNA: Strand breakage was observed for DNA incubated with methyl glyoxal, lysine and ferric chloride for 2 hours at 37 °C (Fig. 5, Lane 2) as compared to control (Fig. 5, Lane 1). The seed extracts (both K1 and K2) caused the reversal of the strand breakage (Fig. 5, Lane 5 & 7). It can be also observed that there was no strand breakage in the presence of solvent methanol (Fig. 5, Lane 3).



Lane Description(10µg)
Lane 1: pBR322 alone
Lane 2: pBR322+ Lysine + MG + $FeCl_3$
Lane 3: pBR322+ methanol
Lane 4: pBR322 + K1(10µg/µL)
Lane 5: Lane 2 + K1
Lane 6: pBR322 + K2(10µg/µL)
Lane 7 : Lane 2 + K2

Fig 5:Analysis of Glycated pBR322 in the presence of Black Cumin Seeds

pBR322 (0.25 µg) was incubated with lysine (20 mM), MG (20 mM) and $FeCl_3$ (100 µM) in the presence or absence of K1 and K2 (10 µg) for 2 hrs at 37 °C. Incubated samples were loaded onto1% Agarose gel. Gel was stained with Ethidium bromide and visualized on a UV transilluminator.

The process of glycation starts with formation of Amadori products which get converted to advanced glycation end products. These AGEs cause structural alteration of biomolecules like nucleic acids and proteins. Reactive oxygen species are generated as a consequence of glycation. The net effect of structural alteration and free radical generation is the loss of activity of biomolecules and results in the emergence of pathophysiological complications. In last several decades' attempts have been made to develop a drug which can prevent the accumulation of AGEs in the body especially for people suffering from Diabetes. Aminoguanidine was one of those artificial drugs which could work as potent antiglycating agent for several years. Later on some side effects of this drug were observed in many patients. Several natural compounds have also been checked for their antiglycating properties. *Nigella sativa* is well known plant and has used for its various medicinal properties since centuries. There are a very few reports in literature regarding the use of this plant for the prevention of glycation (Zafar et al. 2013; Mehmood et al., 2013). However exact mechanism and other molecular details of this prevention is not very clear.

In the present study extracts of *Nigella sativa* were prepared using hot extraction methods and were dissolved in water as well as methanol. Their antiglycating potential was checked on *in vitro* glycation of BSA by fructose. Measurement of extent of browning has been used as a conventional indicator of the process of glycation. It can be seen from the results obtained that both aqueous and methanolic extracts reduced the extent of browning significantly. The amount of early and advanced glycation end products was quantified using established NBT and DNPH methods. Aqueous extract of black cumin seed was more potent in inhibiting the formation of early glycation products as compared to methanolic extract. On the other hand, the extent of carbonyl content, a marker for AGEs, was decreased to the similar extent by both the extracts. In an earlier report Mehmood et al. (2013) have shown that ethanolic extract of black cumin reduced the amount of total AGEs. Glycation also leads to formation of protein cross-linking and aggregates which have been implicated in neurodegenerative disorders. Spectroscopic analysis of glycated sample at the different wavelengths gives an idea of the extent of protein aggregation. A ratio of absorbance taken at 280 and 340 nm is used to calculate the aggregation index. It can be interpreted from the black cumin seed extracts reduced the aggregation index significantly.

The accumulation of AGEs leads to generation of reactive oxygen species *in vitro* and *in vivo*. Macromolecular structures like DNA and proteins are very prone to these free radicals. In the present study the effect of glycation-induced DNA damage was studied in the presence and absence of black cumin seed extracts. Addition of metal ions catalyzed the generation of free radicals and led to enhanced strand breakage of DNA. There was significant inhibition/reversal of DNA damaged by glycation in the presence of black cumin seed extracts.

4. Conclusion

The results presented in this study show that the black cumin seed extracts (aqueous and methanolic) have significant antiglycating potential. However aqueous extract was more potent in preventing the accumulation of glycation products. The results also indicate that seed extracts could prevent the formation of early as well as advanced glycation end products. Previously Zafar et al (2013) have shown that black cumin seeds extract reduced non-enzymatic glycation levels estimated by thiobarbituric acid assay. In another study Losso et al. (2011) have reported the application of thymoquinone, active compound from *Nigella sativa*, in the prevention of AGE formation.

It can be concluded from this study that the aqueous extracts of *Nigella sativa* possess better antiglycating potential than the methanolic extract. The seed extract can be used for preventing glycation-mediated secondary complications of diabetes, glycoxidative damage of DNA as well as protein aggregation mediated neurological disorders. Further studies need to be carried out to understand the mechanism of inhibition of glycation by *Nigella sativa* and identification of bioactive compounds which can be developed as a natural drug to prevent the glycation-mediated complications.

Conflict of Interest Statement

We declare that we have no conflict of interest.

Acknowledgements

The study was funded by Research Society for the Study of Diabetes in India (RSSDI/HQ/Grants/2017/342) and University of Mumbai (grant no. 179) and University Grants Commission, New Delhi (F.20-10(10)/2013(BSR)).

References

Ahmed, N., 2005. Advanced glycation end products-role in pathology of diabetic complications. Diabetes Res. Clin. Pract., 67, 3-21.

Ahmad, S., Moinuddin, S.U., Khan, M.S., Habeeb, S., Alam, K., and Ali, A., 2014. Glyco-oxidative damage to human DNA – Neo-antigenic epitopes on DNA molecule could be a possible reason for autoimmune response in type 1 diabetes. Glycobiology, 24, 281-291.

Ali, A., More, T.A., Hoonjan, A.K., and Sivakami, S., 2017. Antiglycating potential of acesulfame potassium: an artificial sweetener. Appl. Physiol. Nutr. Metab., 42, 10, 1054-1063.

Banan, P., and Ali, A., 2016. Preventive effect of phenolic acids on *in vitro* glycation. Annals of Phytomedicine-an International Journal, 5, 2, 97-102.

Brownlee, M., Vlassara, H., and Cerami, A., 1984. Non-enzymatic glycosylation and the pathogenesis of diabetes complications. Ann. Intern. Med., 101, 527-537.

Friendman, M., 1996. Food browning and its prevention: an overview. J. Agric. Food Chem., 44, 631-653.

Jakus, V., and Rietbrock, N., 2004. Advanced glycation end-products and the progress of diabetic vascular complications. Physiological Research, 53, 2, 131-142.

Khan, M.N., and Gothalwal, R., 2018. Herbal origins provision for non-enzymatic Glycation (NEGs) inhibition. Frontiers in Medicinal Chemistry and Drug Discovery, 2, 1, 10-15.

Kikuchi, S., Shinpo, K., Takeuchi, M., Yamagishi, S., Makita, Z., Sasaki, N., and Tashiro, K., 2003. Glycation - a sweet tempter for neuronal death. Brain Res. Brain Res. Rev., 41, 306-323.

Losso, J.N., Bawadi, H.A., Chintalapati, M. 2011. Inhibition of the formation of advanced glycation end products by thymoquinone. Food Chem. 128, 1, 55-61.

Mehmood, T., Moin, S., Faizy, A. F., Naseem, S., and Aman, S., 2013. *Nigella sativa* as an antiglycating agent for human serum albumin. Int. J. Sci. Res. 2, 4, 25-27.

Najmi, A., Nasiruddin, M., Khan, R.A., and Haque, S.F., 2012. Therapeutic effect of *Nigella sativa* in patients of poor glycemic control. Asian J Pharm Clin Res, 5, 3, 224-8.

Poulsen, M.W., Hedegaard, R.V., Andersen, J.M., Courten, B.de., Bügel, S., Nielsen, J., Skibsted, L.H., and Dragsted, L.O., 2013. Advanced glycation endproducts in food and their effects on health. Food Chem. Toxic., 60, 10-37.

Rahbar, S., and Figarola, J.L., 2003. Novel inhibitors of advanced glycation end products. Arch. Biochem. Biophys., 419, 63-79.

Requena, J.R., Vidal, P., and Cabezas-Cerrato, J., 1993. Aminoguanidine inhibits protein browning without extensive Amadori carbonyl blocking. Diabetes Res. Clin. Pract., 19, 23-30.

Zafar, H., Hussain, F., Zafar, S., and Yasmin, R., 2013. Glycation inhibition by *Nigella sativa* (Linn) – an *in vitro* model. Asian J Agri Biol, 1, 4, 187-189.