

Nigella Sativa as an Antiglycating Agent for Human Serum Albumin



Medical Science

KEYWORDS : *Nigella sativa*, glycation, glucose, human serum albumin

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ABSTRACT

Hyperglycemia and the accumulation of advanced glycation endproducts (AGEs) in tissues and serum have important roles in diabetic complications. Therefore, the identification of anti-glycation compounds is attracting considerable interest. In this study the antioxidant activity of ethanolic extract of Nigella sativa seeds and the interaction of human serum albumin (HSA) with glucose in the absence and presence of extract was studied by tryptophan fluorescence, AGE fluorescence and SDS-PAGE. The results indicate that Nigella sativa extract has significant amount of total phenolic content and reducing power. Furthermore, it also causes conformational changes in HSA, decrease in AGEs formation, increase in tryptophan fluorescence and decreased cross linking of proteins. Hence, it can be deduced that Nigella sativa has a potent antioxidant and antiglycating activity, which can be employed to delay or prevent the secondary complications of diabetes mellitus.

INTRODUCTION

Non enzymatic glycation is a spontaneous post- translational modification of proteins in which reducing sugars bind covalently to the free amino groups of proteins. This process classically proceeds through early and advanced stages. In the early stage sugars react to the amino groups of lysine side chains and the terminal amino groups of proteins to form unstable Schiff bases and through rearrangement, Amadori products. Then this product undergoes a slow, complex series of chemical reactions to form advanced glycation end products (AGEs) [1,19]. AGEs further react with the free amino groups of proteins causing even more post-translational modification of proteins and hence affecting their biological function. In vivo AGEs contribute to the onset of several diseases including diabetic complications among others [5]. Various studies have shown that AGEs have a positive correlation with oxidative stress [14, 16].

Glucose and other reducing sugars have been used in vitro as glycosylating sugars [17]. Thus far some compounds such as amino-guanidine [4], Vit. B6 [3] and quercetin [11] are reported to be inhibitors of glycation reaction. Studies have shown that intake of *Nigella sativa* oil has a beneficial effect on glycemic control in humans [12] as well as in experimental animals [7]. *Nigella sativa* Linn. (Family- Ranunculaceae) is a widely used medicinal herb throughout India, popular in various Indigenous System of medicine like Ayurveda, Siddha, Unani and Tibb. Here, we present our data on *Nigella* as an in-vitro antiglycating and antioxidant agent.

MATERIALS AND METHODS

Nigella sativa ethanolic extract was prepared in the department of pharmacy, Jamia Hamdard, using a Soxhlet apparatus. The yield was 20% w/w.

Determination of Total Phenolic Content:

Total phenolic content was determined by the method of Saucier et al., (1999) [13] with slight modification and the results were expressed directly in absorbance units at 765 nm. Gallic acid was used as a standard for the calibration curve.

Total Reducing Power:

Total reducing power was determined as described by Zhu et al., (2002) [20] with some modifications. *Nigella sativa* extracts in (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mg/ml) 1 ml of

ethanol were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K₃Fe(CN)₆]; the mixture was then incubated at 50°C for 30 minute. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl₃ (0.1%), and the absorbance was measured at 700 nm.

Glycation of HSA

HSA in concentration of 1 mg/ml, glucose in concentration on 50mM and the ethanolic extract of *Nigella sativa* seeds in concentrations of 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml and 1 mg/ml was dissolved in PBS (pH 7.4) and incubated in different containers for 21 days. In one box of each concentration amino-guanidine was also added to the concentration of 0.5nM. After the requisite number of days 5ml of incubate was taken from the box and dialysed to remove excess sugar. Then the protein concentration of incubate was determined using Lowry's method of protein estimation. After this the dialysed incubate was stored in eppendorfs and frozen at -20°C and used for further studies.

Tryptophan specific fluorescence

The fluorescence of tryptophan residue Trp214 in native and glycosylated HSA was monitored with excitation at 285 nm and emission measured over the range of 290-440 nm (Shaklai et al., 1984) [15]. The protein concentration was taken as 100 µM.

Advanced glycation end products (AGEs) specific fluorescence

AGE formation was measured by determining the fluorescence by excitation at 370 nm and emission between 400-500 nm (Galle et al., 1998) [6] using a spectrofluorophotometer. The concentration of protein sample was taken as 100 µM.

1.6. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was performed by the method of Laemmli (1970) [9]. Proteins samples were prepared in the sample buffer containing 10% (w/v) SDS, 10% (v/v) glycerol, 1.0 M Tris HCl, pH 6.8, traces of bromophenol blue as tracking dye and 5% β-mercaptoethanol. The samples were boiled at 100°C for 5

minutes. Then electrophoresis was performed in electrophoresis buffer containing 25mM Tris, 250mM glycine and 0.1 % SDS at a voltage of 50 volts till the tracking dye reached the bottom of the gel.

1.7. Statistical analysis: Data are expressed as mean \pm standard deviation (SD). All experiments were performed independently at least three times. Statistical analyses were performed with one-way ANOVA software.

RESULTS

Total Phenolic Content

The ethanolic extract of *Nigella sativa* seeds was found to have phenolic compounds in the range of 300-350 μ g Gallic acid /g of ethanolic extract of *Nigella sativa* seeds.

Total Reducing Power

A concentration dependent increase in the reducing power of the ethanolic extract of *Nigella sativa* seeds was observed (Fig.1)

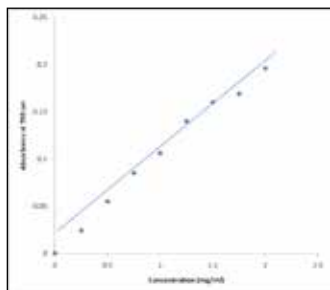


Fig. 1: Total reducing ability of increasing dilutions of ethanolic extract of *Nigella sativa* seeds.

Tryptophan Specific Fluorescence: Glycated HSA showed a decrease in fluorescence activity. After 21 days of glycation with various concentrations of ethanolic extracts of *Nigella sativa* seeds there was seen a drop in the decrease of fluorescence in a concentration dependent manner (Fig.2).

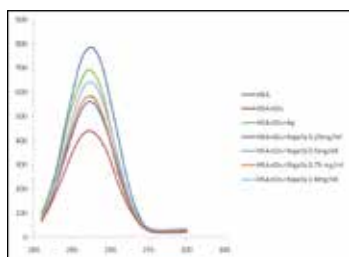


Fig.2: Tryptophan specific fluorescence spectra of native, glycated and *Nigella sativa* extract treated glycated HSA after 21 days of incubation.

1.4.AGE-specific fluorescence

AGE fluorescence was also found to increase in HSA incubated only with glucose. A concentration dependent decrease in AGE fluorescence was observed in HSA samples that were incubated with 4 concentrations (0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1mg/ml) of ethanolic extract of *Nigella sativa* seeds over a period of 21days (Fig.3).

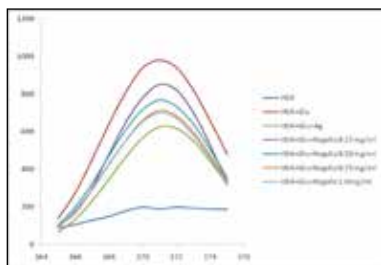


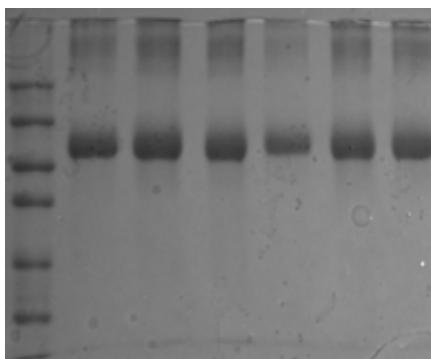
Fig.3: AGEs specific fluorescence spectra of native, glycated and

Nigella sativa extract treated glycated HSA after 21 days of incubation.

1.5.Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

Native HSA showed band of \sim 66 kDa (Fig.4, Lane 2). Glycated and extract treated glycated HSA showed a visible difference in electrophoretic pattern. Glycated HSA showed increase in band intensity as well as broadening of bands towards low as well as high molecular weights, specially high molecular weights which can be attributed to extensive inter and intra molecule cross linking due to glycation, resulting in the formation of high molecular weight aggregates (Fig.4, Lane 3).

Electrophoretic pattern of glycated HSA treated with ethanolic extract of *Nigella sativa* at concentrations of 0.25, 0.5, 0.75, 1.0 after 21 days of incubation showed a reduction in the intensity and broadening of bands showing a decrease in glycation and hence cross linkage and aggregate formation (Fig.4). This inhibitory effect was seen in a dose dependent manner.



Lane 1 Molecular weight standard in kDa

Lane 2 Human Serum Albumin

Lane 3 HSA + Glucose

Lane 4 HSA + Glucose + *Nigella sativa* extract (0.25mg/ml)

Lane 5 HSA + Glucose + *Nigella sativa* extract (0.50mg/ml)

Lane 6 HSA + Glucose + *Nigella sativa* extract (0.75mg/ml)

Lane 7 HSA + Glucose + *Nigella sativa* extract (1.0mg/ml)

Fig.4: SDS-PAGE of HSA upon in vitro glycation in absence and presence of ethanolic extract of *Nigella sativa* seeds after 21 days of incubation.

DISCUSSION

Oxidative stress and non enzymatic glycation, has been known to play an important role in a large number of diseases including complications of Diabetes mellitus among others[5].

Thus far some drugs such as aminoguanidine[4], aspirin and ibuprofen are reported to be inhibitors of glycation reaction. But these drugs have certain limitations due to their toxicity. Similarly many natural products eg. Quercetin have an inherent antiglycating and antioxidant property. Rutin from tomato paste is a potent antiglycating agent and also acts as an antioxidant in vitro [8]. Ascorbic acid and other vitamins and nutrients have been shown to act as potent antioxidants as well as possible inhibitors of protein glycation and AGE formation in vitro [18]. Phenolic compounds from Louboma tea have been shown to be more potent than aminoguanidine in inhibiting glucose mediated glycation and formation of AGEs in vitro. Aged garlic extract and its active ingredient S-allyl cysteine inhibits AGE formation in vitro. They suppress AGE formation by preventing glyoxidation and sequestering the 1,2- dicarbonyls, that are an important precursor of AGEs. Phenols exert their antioxidant effects by transferring a hydrogen atom to the chain-carrying ROO• radicals.

Nigella sativa Linn.(Family- Ranunculaceae) is a widely used medicinal herb throughout India, popular in various Indigenous System of medicine like Ayurveda, Siddha, Unani and Tibb. The total phenolic content was found to be 300-350 μ g Gallic acid/g

of ethanolic extract of *Nigella sativa*. A concentration dependent increase in total reducing power was also observed. The antioxidant activity can be attributed to the polyphenols, tocopherols as well essential oils such as thymoquinone [2].

Furthermore we also studied the antiglycating activity of the extract. For this we incubated HSA with Glucose and with four different concentrations of the extract. *Nigella sativa* inhibits the non enzymatic glycation of albumin in vitro in a concentration dependent manner. A decrease in glycation of HSA and AGEs formation was demonstrated by SDS PAGE, Tryptophan fluorescence and AGE fluorescence. The antiglycating activity of the ethanolic extract of *Nigella sativa* can be explained in relation to the antioxidant activity. The antioxidant activity of *Nigella sativa* prevents autoxidation of glucose which would otherwise result in formation of more enediol radicals which would fur-

ther be oxidized to form dicarbonyl ketoaldehyde which reacts with amino groups in proteins to form a ketoamine. Ketoamines participate in AGE formation [5]. They are similar to Amadori products albeit more reactive than them. These findings though prove the efficacy of the ethanolic extract of *Nigella sativa* as an antioxidant as well as an antiglycating agent.

CONCLUSION

Our findings suggest the efficacy of the ethanolic extract of *Nigella sativa* as an antioxidant as well as antiglycating agent as it is a rich source of polyphenols.

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