GLYCATION INHIBITION BY *NIGELLA SATIVA* (LINN)–AN *IN VITRO* MODEL

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**ABSTRACT**

In present study, an *in vitro* glycation model comprising both plasma and glucose designed to assess glycation inhibitory potentials of *N. sativa* seed (NS) extracts. Plasma with several glucose quantities in the presence of NS were incubated for thirty-five days at 37°C. Non-enzymatic glycation (NEG) was quantified by Thiobarbituric acid technique. NEG levels were reduced by NS. Higher NS concentrations significantly (P < 0.05) reduced the NEG demonstrating dose-dependent inhibitory potential of NS. Plasma NEG levels were decreased up to 13% (P< 0.05). NEG inhibition correlated (r=0.784) with NS concentrations. Present glycation inhibitory prospective of NS demands further studies to determine the precise mechanism of action and effective dose of NS for therapeutic interventions.

**Keywords**: Glycation inhibition, Hyperglycemia, Human plasma, *Nigella sativa*

**INTRODUCTION**

Diabetes mellitus (DM) is a group of metabolic disorders affecting millions worldwide (Uma and Sudarsanam, 2012). Hyperglycemia has a key role in non-enzymatic glycation (NEG) of lipids, proteins and nucleic acids. Diabetic complications are greatly enhanced with increase NEG and oxidative stress (Wu et al., 2011). In this regard, several natural compounds known to possess antioxidative and antiglycation properties, such as curcumin, rutin, garcinol and flavonoid-rich extracts, have been shown to prevent glycation formation *in vitro* and *in vivo*. These agents that inhibit the formation of protein glycation are purported to have therapeutic potentials in patients with diabetes and related diseases (Kim and Kim, 2003; Akash et al., 2011; Mathur et al., 2011). *Nigella sativa* (family Ranunculaceae) is generally known as “black cumin.” *Nigella sativa* seed (NS) is used as a flavoring spice and for the management of diabetes mellitus, infection and cancer. *N. sativa* may be beneficial in diabetic individuals and those with glucose intolerance as it reduces appetite, intestinal glucose absorption, hepatic gluconeogenesis, blood glucose level, cholesterol, triglycerides, body weight and simulates glucose induced secretion. In streptozotocin (STZ) induced diabetic rats it caused partial regeneration of pancreatic beta-cells, increased the lowered serum insulin concentrations and decreased the elevated serum glucose. *N. sativa* has antioxidant activity and protective role against development of diabetes mellitus (Mathur et al., 2011; Javed et al., 2012). However, limited data is available on antiglycation activity of NS. Therefore, the present study was conducted to assess *in vitro* protein glycation inhibitory activity of *Nigella sativa*.

**MATERIALS AND METHODS**

**Extract preparation**

NS were washed with distilled water, air-dried and ground into powder. NS powder mixed with distilled water was kept on orbital shaker for 48 hours at 120 rpm and room temperature. Mixture was filtered and filtrate was evaporated to dryness. Before analysis, it was reconstructed as I₁ (500 mg/mL), I₂ (250 mg/mL) and I₃ (50 mg/mL) (Mensor et al., 2001).

**Collection of Samples**

Healthy normoglycemic volunteers provided blood samples after consent. Plasma was separated and all the plasma samples were pooled together.

**Incubations**

Plasma samples were incubated with glucose (5.5, 25 and 50mM) and NS extracts (50, 250, 500 mg/ml) in phosphate buffer saline (PBS)
at 37°C for five weeks (Table 1). Glucose was used as glycation agent. While, NS acted as an inhibitor. At the end of incubation period, glucose concentrations were measured. Another glucose measurement was done after dialysis to validate final glucose levels. Total protein bioassay was conducted by Biuret method (Gornall et al., 1949).

**Glycation analysis**

Glycation was determined by TBA (Thiobarbituric acid colorimetry) test (Furth, 1988).

1. Glycation (NEG + EG)

One ml sample (after dialysis and protein levels as 10 mg/ml) was used. Three test tubes were arranged for reduced and three for non-reduced samples. NaBH₄ and 0.01N NaOH (0.1ml each) was used for reduced and non-reduced samples respectively. After incubation (37°C, 30 minutes), 10 µL of 1N HCl, 0.5 ml oxalic acid were added and autoclaved at 124°C (115 Lb/inch² pressure, 30 minutes). After cooling, 40% trichloroacetic acid (0.5mL) was added and centrifuged (5000 rpm) for 15 minutes. Supernatant (1500µL) was mixed with same amount of TBA. After incubation at 37°C (15 minutes), absorbance was taken at 443 nm (Furth, 1988).

2. Enzymatic Glycation (EG)

For EG, 0.1 ml NaOH (0.01N) with excess of NaBH₄ was used. The glycation level was determined by TBA test (Furth, 1988).

3. Non-enzymatic glycation (NEG)

Non-enzymatic glycation was determined (Furth, 1988) as follows:

\[ \text{NEG} = (\text{NEG} + \text{EG}) - \text{EG} \]

All data were expressed as mean ±SE of triplicate measurements. Statistical analyses was performed by SPSS software (version 15.0) with level of significance set at \( p < 0.05 \).

**RESULTS**

Results of the present study are presented in figure 1. BSA was used as negative control. Normal plasma (PN+G₁, PN+G₂, PN+G₃ as positive control) incubated with 50, 25 and 5.5mM glucose had 4.07 ± 0.068, 3.65 ± 0.017 and 3.09 ± 0.05 (mole glucose/mole protein) NEG levels. By using I₁, NEG was significantly (\( p < 0.05 \), 7.11-13%) decreased as compared to positive controls. Decline in NEG remained significant (\( p < 0.05 \), 3.23-9.33%) when diluted inhibitor concentration (I₂) was used. Although, I₁ (50 mg/ml) was 10 times diluted as compare to the I₁, it had effective (\( p < 0.05 \), 8.1%) inhibitory activity. Results indicated dose-dependent inhibitory potential of NS, as I₁ halted NEG more efficiently as compared to I₂ and I₃. Similarly, G' generated maximum NEG as compared to other glucose concentrations (G₂, G₃). NEG inhibition correlated (\( r=0.784 \)) with NS concentrations.

**DISCUSSION**

The non-enzymatic glycation or maillard reaction is associated with diabetes related complications. The investigation of natural glycation inhibitors may provide new avenues for drug discovery (Wu et al., 2011). The effect of NS on glycation induced damage is never studied. In the present study, plant extracts demonstrated antiglycation activity when tested by plasma-glucose assay. The mechanism of NS antiglycation activity was not explored in the present study. Glycation inhibitory activity of NS may be due to different phenolic components. Numerous studies have shown that many herbs and spices have high polyphenol content and antiglycation activities. Glycation inhibition by plants can be due to their antioxidant activity or by a carbonyl blocking mechanism which prevents the formation of amadori products (Kim and Kim, 2003; Lunceford and Gugliucci, 2005; Thomas et al., 2005). Wu et al., 2011 investigated the inhibitory effect of naturally occurring flavonoids on protein glycation using in vitro models. The results indicated that protein glycation inhibitory capability of flavonoids was remarkably related to the scavenging free radicals derived from glycoxidation process. Our data is partially in accordance with the Losso et al., (2011). They demonstrated that thymoquinone,a major quinone from *Nigella sativa* has an inhibitory action against the glycation process. At 20 µM, thymoquinone inhibited 39% of hemoglobin glycation. Earlier, in vitro glycation was studied by Vinson and Howard (1996) with BSA (bovine serum albumin) with glucose or fructose as glycating agents. Eighteen normal subjects were supplemented with 1000 mg of ascorbic
acid for a period of 4 weeks. Serum protein glycation was decreased an average of 46.8% (p < 0.01). These results underline the importance of nutrition in diabetes and indicate the possibility of therapeutic use of these nutrients for the prevention of diabetic complications.

Present study demonstrates the possibility of inhibiting NEG with NS seeds extracts in vitro. Furthermore, long-term in vivo studies are needed to demonstrate the usefulness of NS supplementation for the prevention of diabetic complications.

CONCLUSION

NS was demonstrated to possess antiglycation activity based on an in vitro glycation model. However, the mechanism of NS antiglycation activity and its application as a therapeutic agent need further investigation. It is hoped that such therapies will be fully evaluated in the clinical context with the ultimate aim to reduce the major economical and medical burden of diabetes mellitus.

REFERENCES


