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Original Article

Free Radical Scavenging and Cyto-protective Activity of Ethanolic Extract of Nigella Sativa Seeds

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ABSTRACT

Introduction: *Free radicals and the oxidative stress have been implicated in a large number of chronic disorders such as Diabetes mellitus and its late complications, Cardio vascular disease, Arthritis also in some acute conditions such as the hemolytic disease in Glucose 6 Phosphate Dehydrogenase (G 6 PD) deficiency, where free radicals play a direct cytotoxic role causing cellular damage. Various exogenous substances have been found to be of great use for the purpose of scavenging free radicals. These includes micro nutrients such as vitamins eg. Vit C, Vit A etc or other dietary agents polyphenols, flavenols, tannins etc. In traditional medicine certain food items and their extracts are considered useful in combating conditions such as diabetes mellitus, Cardio vascular diseases etc and their long term complications that are caused by oxidative stress. Nigella Sativa seeds are one such condiment used in food in south east, central Asia and middle east and also used in ancient Indian (Ayurveda) and Greeko-arabic (Unani) systems of medicine.* ***Material and Method***: *The objective of this study is to quantify the free radical scavenging and Cytoprotective effects of ethanolic extract of Nigella Sativa seeds. To measure the free radical scavenging activity DPPH free radical scavenging assay was used. To measure cyto-protective effects of Nigella Sativa seed extract, an AAPH assay was used with the Cyto-protective effect being measured on RBCs (Red blood cells) suspended in PBS buffer.* ***Results***: *In the DPPH assay the ethanolic extract of Nigella Sativa seeds showed significant free radical scavenging activity. The activity was concentration dependent.* ***Conclusion***: *In AAPH RBC lysis assay the ethanolic extract of Nigella Sativa seeds did show considerable protective effect against AAPH induced RBC lysis. Once again the activity was concentration dependent.*

Key words: *Nigella Sativa, Antioxidants, Free radical scavengers.*

INTRODUCTION

Oxidative stress has, in recent years, become a major point of interest in study of the pathogenesis of chronic and degenerative disorders such as Diabetes mellitus and its late complications,[1,2] cardio vascular diseases,[3,4] Alzheimer's disease,[5,6] Parkinson's disease[7,8] and other disorders of aging conditions such as Glucose 6 phosphate dehydrogenase deficiency show a much more dramatic effect of free radical injury. Imbalance between the pro and anti-oxidant forces cause damage to cellular membrane causing RBC lysis and RBC sequestration. Rarely dramatic massive hemolysis is seen during an oxidative crises in a G6PD deficient individual.[9-12]

With increased implication of Free radical damage and oxidative stress in pathophysiology of various disease there has also been a rising interest in anti-oxidants. Many endogenous antioxidants are present in our cells ,to maintain a healthy oxidant antioxidant balance, such as superoxide dismutase, glutathione peroxidase, lipoic acid, uric acid etc. Apart from these a large number of exogenous antioxidants from natural sources such as polphenols, tannins, flavanols, non-flavanoid phenols, Vitamins such as vitamin E, vitamin C have been under study for their anti-oxidant potential. Red wine, green tea

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extracts, various fruits such as sea buckthorn (Hippophae rhamnoides), aged garlic extract have shown potent antioxidant potential.[13-16]

Nigella Sativa is a small herb, the seeds of which are used as a condiment in Middle Eastern and Asian cooking. It's seeds, also known as onion seeds, are also used for its medicinal properties in the ancient Indian (Ayurevedic) and ancient Greeko-Arabic (Unani) system of traditional medicine as a remedy of a multitude of disorders including Diabetes and its related complications.

The fats present in the seed are considered to play an important role in its therapeutic activity. Oils constitute 40.35(± 0.06)% of dry matter. Among the Fatty acids the predominant are Linoleic acid, 49.15 (±0.06) gm/100 of total Fatty acids (FA). It is followed by Oleic acid,23.7 (±0.06) gm/100gm of F.A. and Palmitic acid, 18.4 (±0.06)gm/100gm F.A. Saturated fatty acids make up 25.5 (±0.69)% of total fatty acids. Mono Unsaturated and Poly Unsaturated make up 25.0 (±0.58)% and 49.8 (±0.20)% respectively.[17] Essential fatty acids (EFAs) form almost 50% of all fatty acids. The high percentage of unsaturated fatty acids and EFAs explain some of the therapeutic effects of Nigella seeds. Essential Oils constitute 0.4 to 2.5% of the Oils obtained from Nigella Sativa seeds. Many components have been characterised in the essential oils but the predominant among them is Thymoquinone, constitutes between 27.8% to 57% of the essential oil content (Ali and Bluden 2003.[18] Thymoquinone and a related synthetic compound tert-butylhydroquinone show potent superoxide ion scavenger activity.[19] Nigella Sativa is also a rich in phenols and polyphenols with the ethanolic extract showing total phenolic content of 31.15±0.29 mg Gallic acid equivalent/gram.[20]

MATERIALS AND METHODS

Preparation of Nigella Sativa extract:

Nigella sativa ethanolic extract prepared by Dr. Mohd Yusuf using a soxhlet apparatus using 95% ethanol as extract solvent. The seeds were sourced from Iran. The yield was 20% (w/w).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging Activity Assay (DPPH) assay:

The free radical scavenging activity of the Nigella Sativa seed extract and standard reference compound i.e, Gallic acid was analysed by the DPPH assay[21] with minor modification. In this assay, 1 ml of varying concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mg/ml) of ethanol extract of Nigella Sativa, dissolved in 1 ml of ethanolic solution of DPPH (0.2 mM). The mixture was vortexed and incubated for 30 min. The optical density of the solution was the measured at 517 nm using Hitachi 2010 spectrophotometer. Gallic acid (µg/ml) has been used as a comparison. The DPPH radical scavenging activity was calculated from the absorption according to the following equation:

$$Radical\ scavenging\ activity\ (\%) = [(O.D._{Control} - O.D._{Sample}) / O.D._{Control}] \times 100$$

2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) RBC lysis assay:

In this study,[22] the method described was used to determine erythrocyte haemolysis mediated by AAPH. Blood was obtained from healthy human donor and collected into heparinized tubes through the Blood Bank, J. N. Medical College, Aligarh Muslim University, Aligarh. Erythrocytes were separated from plasma and the buffy coat, and washed three times with 5 volumes of phosphate buffered saline (PBS), pH 7.4. During the last wash, the erythrocytes were centrifuged at 3000g for 10 min to obtain a packed cell preparation. The packed erythrocytes were then suspended in 4 vol of PBS solution. Two milliliters of the erythrocyte suspension was mixed with 2 ml of PBS solution containing varying amounts of ethanolic extracts of Nigella Sativa (.250, .5, .75, 1.0, 1.25, 1.5, 1.75, and 2.0 mg/ml). Two milliliters of 200 mM AAPH in PBS was then added to the mixture in induce free radical induced hemolysis. The reaction mixture was shaken gently while being incubated at 37°C for 3 hr. After incubation, the reaction mixture was diluted with 8 Vol of PBS and was centrifuged at 3000g for 5 min. The absorbance (A) of the supernatant fraction at 540 nm was recorded in a spectrophotometer. The zero was set using a supernatant from a RBC PBS suspension. The percentage of inhibition was calculated by the following equation:

$$\%\ Inhibition = [A_{AAPH} - A_{Extract}] / A_{AAPH}$$

Where $A_{Extract}$ is the absorbance of the sample containing different amounts of Nigella sativa extract, and AAAPH is the absorbance of A_{AAPH} at 540 nm. Gallic acid was used as a positive control. Experiment was performed in triplicate and the average values were taken. The whole experiment was repeated at least three times on different days.

Graphs and statistical analysis: Ms Excel was used to plot the graphs

RESULTS

DPPH assay: The ethanolic extract of Nigella sativa was found to have a good free radical scavenging activity. The radical scavenging activity was concentration dependent as seen by using serial dilutions in the assay (Fig. 1). 50% inhibition of the free radicals generated in the assay was achieved at a concentration of 0.89 mg/ml (890µg/ml). In a similar assay using gallic acid, as a free radical scavenger, 50% inhibition was obtained at a concentration of 3.7µg/ml (Fig. 2).

AAPH RBC lysis assay: The incubation of RBCs with different concentrations of ethanolic extracts of Nigella Sativa seeds showed a potent protective effect against AAPH free radical induced RBC lysis. This protective effect also showed an increase in concentration dependent manner (Fig. 3). 50% inhibition of RBC lysis in the assay was attained at a concentration of 0.55mg/ml (550µg/ml). In a similar assay using Gallic acid as a free radical

scavenger 50% inhibition of RBC lysis in the assay was achieved at a concentration of 80µg/ml (Fig.4).

DISCUSSION

Nigella Sativa seed extract has shown Cyto-protective effect in a large number of studies. Al Mofleah[23] studied the protective effects of aqueous suspension of Nigella Sativa on the gastric mucosa and showed that Nigella Sativa protected the gastric mucosa of Wistar albino rats against ethanol induced damage. A large number of studies have proven the hepatoprotective effect of Nigella sativa seed extract. Ghadlinge[24] reported hepatoprotective effect of Nigella sativa seed oil against Acetaminophen induced hepatotoxicity in albino rats. Kanter et al[25] reported the hepatoprotective effect of Nigella Sativa against in carbon tetrachloride treated rats.

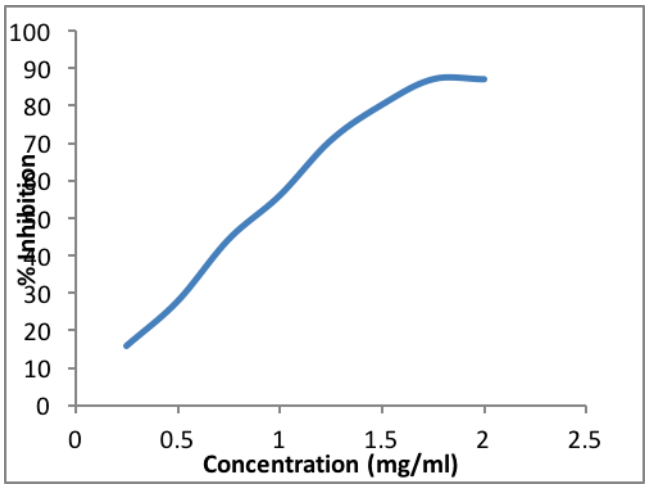

Fig.1: Percentage inhibition of DPPH radicals by increasing concentrations of ethanolic extract of Nigella sativa seeds.

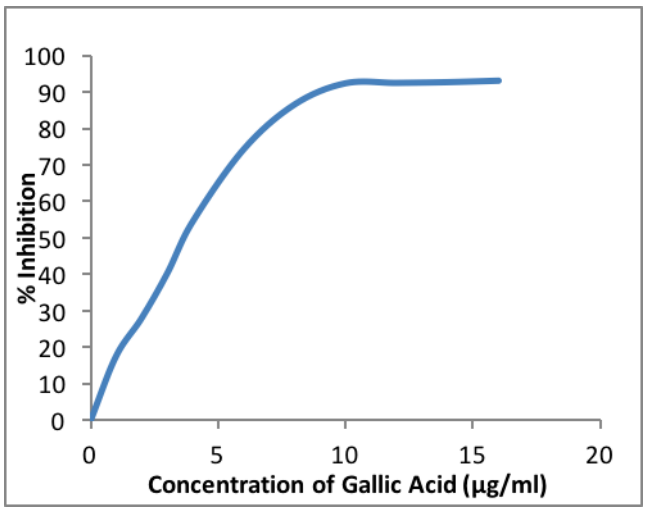

Fig. 2: Percentage inhibition of DPPH radicals by increasing concentrations of Gallic acid.

Rehman et al 2007[26] displayed the Cyto-protective effect of Nigella sativa on the renal tubules against Gentamycin induced renal toxicity. In this study we had undertaken two main exercises. In the first exercise we have studied the antioxidant as well as the free radical scavenging effect of the ethanolic extract of Nigella sativa using DPPH assay. The second exercise we have measured direct Cytoprotective effect of Nigella Sativa seed extract on RBCs in AAPH RBC lysis assay.

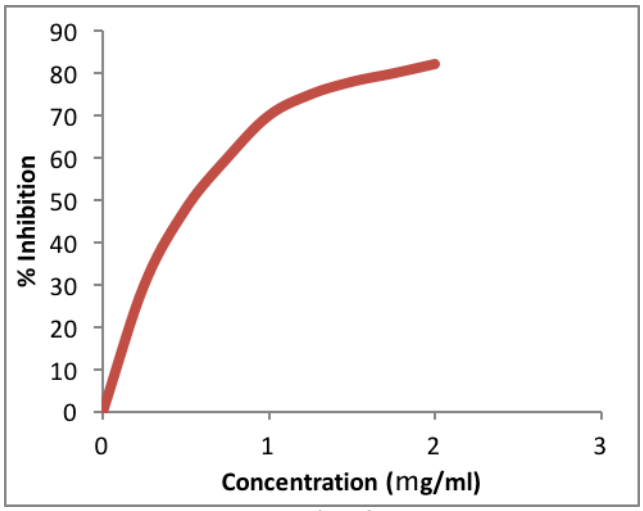

Fig.3: Percentage inhibition of RBC lysis in AAPH assay by increasing concentrations of ethanolic extract of Nigella sativa seeds.

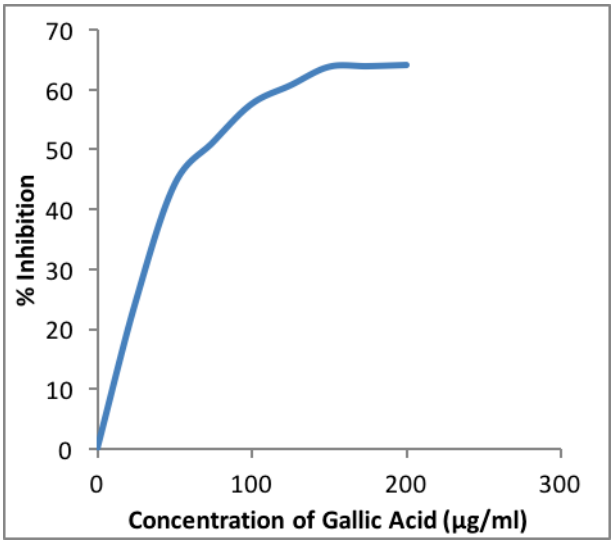

Fig. 4: Percentage inhibition of RBC lysis in AAPH assay by increasing concentrations of Gallic acid.

The ethanolic extract of Nigella Sativa seed showed potent antioxidant and free radical scavenging activity in the DPPH assay. In DPPH assay it showed increase in free radical scavenging activity in a concentration dependent manner. 50% inhibition of DPPH free radical was obtained at a concentration of 0.89 mg/ml. In AAPH RBC lysis assay the ethanolic extract of Nigella Sativa exerted a Cyto-protective effect in a concentration dependent manner, preventing RBC lysis. 50% inhibition of RBC lysis was achieved at a concentration of 0.55mg/ml. There is a parallel between the anti-oxidant activity of Nigella Sativa seed ethanolic extract and its Cyto-protective activity against APPH free radical induced RBC lysis. This gives us reason to believe that the Cyto-protective activity of the extract is due to its free radical scavenging activity.

Karimi et al 2011[27] have also reported protective effect of ethanolic and aqueous extracts of Nigella Sativa seeds against free radical induced RBC lysis. This effect may be due to the glutathione sparing effect of Nigella Sativa

extract as reported by El-Sayed 2011[28]. This Glutathione sparing effect may be due to the chain breaking free radical scavenging effect of the phenols present in the ethanolic extract of Nigella Sativa seeds (Burkit and Duncan 2000)[29] (Kowalewska and Litwinienko 2010)[30]

The Essential fatty acids also have a protective effect against free radical induced injury in cell membranes (Zararsiz et al 2006).[31] Kırmızıgül et al[32] in their study on Cephalaria (Dipsacaceae) species have shown a significant positive correlation between Essential fatty acid content and anti-oxidant activity. This should stand true for Nigella Sativa too which has a high content of essential fatty acids, nearly 50%, and this would also explain a large number of the therapeutic effects of Nigella Sativa seed extracts.

CONCLUSION

Keeping these facts in mind Nigella Sativa extract has a role to play as a Cyto-protective agent in various diseases. Its protective effect against RBC lysis in face of oxidative stress can have a role in disorders like Glucose 6 Phosphate dehydrogenase deficiency where the primary pathology is damage to RBC membrane due to oxidative insults.

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