

Full Length Research Paper

## Down-regulation of GST and CAT gene expression by methanolic extract of *Nigella sativa* seed in human peripheral blood mononuclear cells

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The anti-oxidant effect of *Nigella sativa* (NS) on human Peripheral Blood Mononuclear Cells (PBMCs) on a PHA (phytohemagglutinin) and a non-PHA stimulated proliferation were tested using SYBR Green based Real Time PCR analysis for antioxidant enzyme's gene expression [glutathione-S-transferase (GST) and catalase (CAT)]. Cells isolated from human PBMCs were treated with methanolic extract of NS for 48 h in two separate environments (PHA and non-PHA stimulated). The results obtained indicate that extracts from NS had down regulation effect. GST was down regulated to 1 and 1.5 fold after exposure to 2.5 µg/L NS extract for 48 h in PHA stimulated cells compared to respective controls whereas it decreased to 4 and 2 fold at dose of 5 µg/L in non-PHA stimulated cells compared to respective controls. Similarly, CAT was down regulated to 2 and 6 fold after exposure to 2.5 µg/L NS extract for 48 h whereas, it decreased to 4 and 2 fold at dose of 5 µg/L in PHA and non-PHA stimulated cells compared to respective controls. This *in-vitro* study reveals the effects of NS plant extract on GST and CAT gene expression in human PBMCs.

**Key words:** *Nigella sativa* methanolic extract, glutathione-S-transferase (GST), catalase (CAT), oxidation, peripheral blood mononuclear cells.

### INTRODUCTION

*Nigella sativa* (NS) seeds, a natural food additive and a condiment are consumed by mixing with honey and in baking products or pastries. NS seeds had been used for medicinal uses as a natural remedy in many ancient cultures including Egypt, Greece and Rome (Al-Hader et al., 1993). NS seeds are a good source of essential fatty acids (Al-Ghamdi, 2003), as well as antioxidants (Salem, 2005). Polyunsaturated fatty acids account for the largest fraction followed by linolenic, linoleic and palmitic acids and many other fatty acids (Cheikh-Rouhou et al., 2007). NS is a rich source of vitamin A1, B1, B2, C and niacin as well as K, P, Ca, Mg, Na, Fe, Zn, Mn, Se and Cu (Salem, 2005; Atta, 2003). Oxidative stress is recognized as one

of the major contributors for the increased risk of chronic diseases such as cancer and cardiovascular diseases (Moylan and Reid, 2007). NS has clear antioxidant properties and can be used as an antioxidant against oxidative stress (Sen et al., 2010; Yoruk et al., 2010). NS seeds are good source of natural antioxidants such as selenium, thymoquinone, vitamins E and A. Moreover, NS oil has been an antioxidant activity (Machmudah et al., 2005). One of the potential properties of NS is the ability of its constituents to reduce toxicity due to its antioxidant activities (Yoruk et al., 2010). The consequences of consuming high levels of antioxidants, even naturally occurring ones, over the long period of

time are not clear. Consuming supplements risk toxicity and the higher the dose, the greater the risk of harm. To prove a clear benefit from taking antioxidant supplements, it would be irresponsible for health care professionals to make such recommendations.

One of the potential properties of NS is the ability of one or more of its constituents to reduce toxicity due to its antioxidant activities. However, the pharmacological dose of NS induces apoptosis by promoting pro-apoptotic genes (unpublished data). These pro-apoptotic genes may regulate antioxidant enzyme gene. Therefore, the aim of this study is to investigate the effect of NS seeds extract on the expression of *GST* and *CAT* gene in human PBMCs in the presence or absence of PHA.

## MATERIALS AND METHODS

### Preparation of plant extract

Plant material was pulverized using a milling machine and extracted with methanol using a Soxhlet apparatus as described previously (Shafi et al., 2009). The organic phase was evaporated under reduced pressure to obtain a residue. The residue was dried using a rotary evaporator to obtain the powder/paste. The required quantity of the dry powder/paste was dissolved in dimethyl sulfoxide (DMSO).

### Preparation of peripheral blood mononuclear cells (PBMCs)

The peripheral blood mononuclear cells (PBMCs) were separated from the whole blood of healthy donors by Ficoll-Hypaque gradient centrifugation. The PBMCs were prepared under sterile conditions in RPMI-1640 medium containing 10% fetal calf serum. Their viability, as determined by the trypan blue exclusion test was more than 98%, and their concentration was finally adjusted to  $2 \times 10^5$  cells/ml.

### Lymphocyte assay

100  $\mu$ l of cell suspension were pipetted into each well of 96-well tissue culture plates, to which 100  $\mu$ L of media containing different concentrations of methanol extracts (2.5 and 5.0  $\mu$ g/ml), and 10  $\mu$ L (20  $\mu$ g/ml) of media containing a sub-optimal concentration of phytohemagglutinin (PHA) to stimulate T-cells were added or with PHA (5  $\mu$ g/ml). One triplicate series of wells was used as a negative control (without extracts and PHA). The plates were incubated for 2 days at 37°C in a 5% CO<sub>2</sub> incubator.

### c-DNA synthesis and quantitative RT-PCR analysis

PBMCs were treated with two different concentration of methanolic extract of NS seed and the procedures of quantitative RT-PCR analysis were carried out according to the manufacturer's instructions. Briefly, cDNA was directly prepared from cultured cells by using Cell-to-cDNA synthesis reagent kit (Promega) following the manufacturer's instructions. Reverse transcription-PCR (RT-PCR; Applied Biosystems 7500 Fast, Foster City, CA) was used to analyse the expression of *CAT*, *GST* and *GAPDH* (reference gene) genes mRNA. SYBER Green based primer assays (QIAGEN, Germany), namely *Hs\_CAT\_1\_SG* QuantiTect Primer Assay and *Hs\_GSTA4\_1\_SG* QuantiTect Primer Assay and

QuantiTect Primer Assay were used to analyse the expression of *CAT*, *GST* and *GAPDH* genes. Quantitative real-time RT-PCR was performed in a reaction volume of 25  $\mu$ l according to the manufacturer's instructions. Briefly, 12.5  $\mu$ l of master mix, 2.5  $\mu$ l of primer assay (10x) and 10  $\mu$ l of template cDNA (100  $\mu$ g) were added to each well. After a brief centrifugation, the PCR plate was subjected to 35 cycles of the following conditions: (i) PCR activation at 95°C for 5 min, (ii) denaturation at 95°C for 5 s and (iii) annealing/extension at 60°C for 10 s. All samples and controls were run in triplicates on an ABI 7500 Fast Real-time PCR system. The quantitative RT-PCR data was analyzed by a comparative threshold (Ct) method, and the fold inductions of samples were compared with the untreated samples. *GAPDH* was used as an internal reference gene to normalize the expression of the apoptotic genes. The Ct cycle was used to determine the expression level.

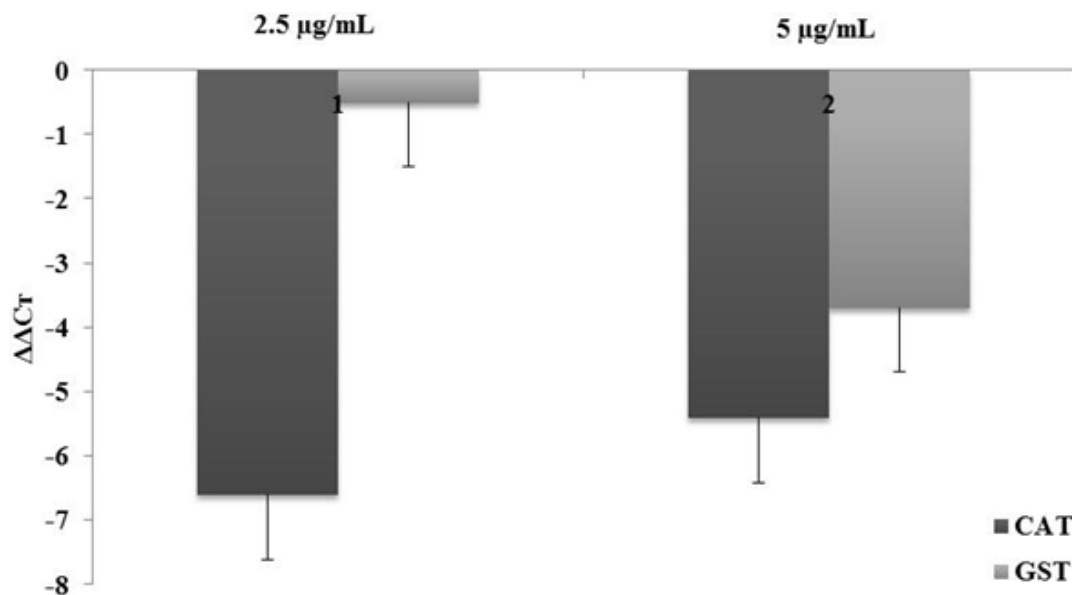
The amount of gene expression was then calculated as the difference cycle threshold ( $\Delta$ Ct) between the Ct value of the target gene and *GAPDH*.  $\Delta\Delta$ Ct is the difference between the  $\Delta$ Ct values of the reference and the target.

### Statistical analysis

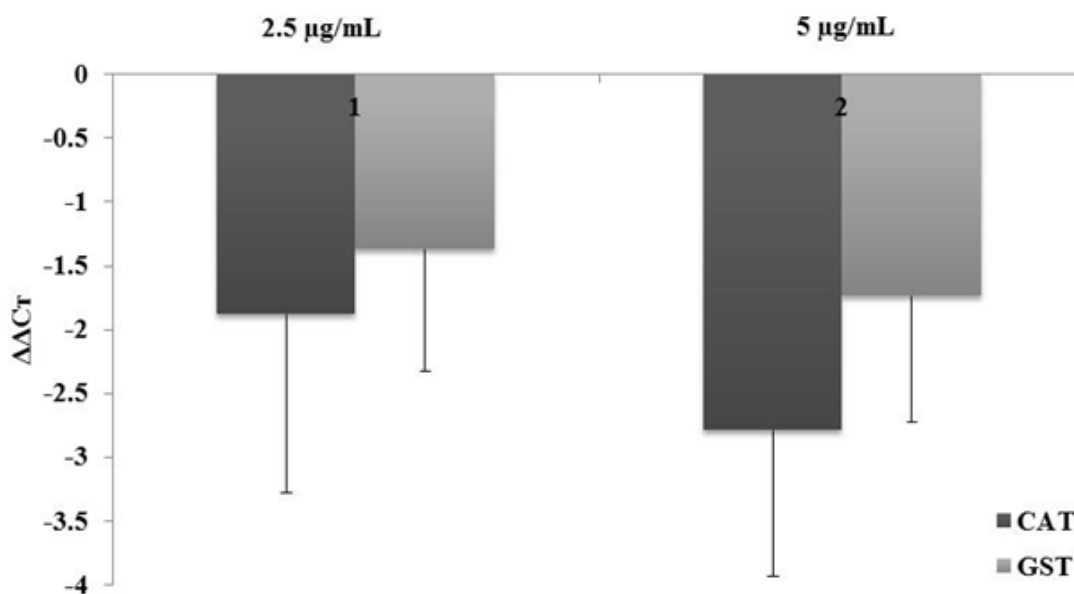
The results of each series of experiments (performed in triplicates) are expressed as the mean values  $\pm$  standard deviation of the mean (SD).

## RESULTS AND DISCUSSION

Antioxidants compounds behave differently at various dose intakes. At physiological levels, they act as antioxidant, whereas at pharmacological levels, some antioxidants act as pro-oxidants, stimulating the production of free radicals, especially when metal ions such as iron are present (Gulcin, 2010; Halliwell, 1994; Repka and Hebbel, 1991; Brown et al., 1997). In order to find the expression of antioxidant genes, *CAT* and *GST* in human PBMCs were analyzed with SYBR Green based real-time quantitative PCR of RNA derived from PBMCs cultured for 48 h in the presence or absence of PHA with methanolic extract of *N. sativa* seed. The results obtained indicate that extracts from NS had down regulation effect. *GST* was down regulated to 1 and 1.5 fold after exposure to 2.5  $\mu$ g/L NS extract for 48 h in PHA stimulated cells compared to respective controls whereas, it decreased to 4 and 2 fold at dose of 5  $\mu$ g/L in non-PHA stimulated cells compared to respective controls. Similarly, *CAT* was down regulated to 2 and 6 fold after exposure to 2.5  $\mu$ g/L NS extract for 48 h whereas, it decreased to 4 and 2 fold at dose of 5  $\mu$ g/L in PHA and non-PHA stimulated cells compared to respective controls (Figures 1 and 2). This study was carried out to investigate the effect of NS seeds extract on the expression of antioxidant genes, *CAT* and *GST* in human PBMCs. Interestingly, the expression of *GST* and *CAT* gene in human PBMCs in the presence or absences of PHA were significantly decreased. These observations are in disagreement with others' who demonstrated that NS increased the antioxidant activities *in vivo* (Bayrak et al., 2008; Morsi, 2000).



**Figure 1.** Expression of genes GST and CAT, in PBMCs treated with methanolic extract of NS at 2.5 and 5 µg/ml in non-PHA stimulated cells compared to respective controls.



**Figure 2.** Expression of genes; GST and CAT, in PBMCs treated with methanolic extract of NS at 2.5 and 5 µg/ml in PHA stimulated cells compared to respective controls.

The biological and pharmacological effects of certain compounds should depend upon its behavior as either an antioxidant or pro-oxidant (Leung et al., 2006). Thymoquinone (TQ), one of major compound present in NS was able to produce significant reductions in hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (Yaheya and Ismail, 2009; Badary et al., 2000). Additionally, luteolin

and flavonoid structures increased SOD and catalase activity over the first 16 h of luteolin treatment, and declined after 24 h luteolin-induced CH27 cell. Apoptosis are closely associated with antioxidant properties of the luteolin. The biological and pharmacological effects of a flavonoid compound should depend upon its behavior as either an antioxidant or prooxidant luteolin (Leung et al., 2006). NS may induce apoptosis by inducing Fas

expression, which is one of the mechanisms of cisplatin killing the malignant cells. For instance, cisplatin could up-regulate expressions of Fas and FasL, activate caspase 8 pathways and promote apoptosis in uterine cervix cancer cells (Toyozumi et al., 2004). Importantly, over-expression of Fas in H446/CDDP cells significantly decreased the expressions of GST at mRNA and protein levels, and increased the cell apoptosis (Wu et al., 2010). Over-expression of pro-apoptotic genes may down regulate the expression of GST and CAT gene. Additionally, the different active components of *N. sativa* could regulate gene regulation of GST and CAT.

Down regulation of GST and CAT genes possibly could be by over expression of Fas which in turn down regulates the expression of GST at mRNA and protein level as reported earlier (Wu et al., 2010). Therefore, it could be concluded from the present study that the methanolic extract of NS seeds may act as pro-oxidants by reducing the expression of GST and CAT gene. In a nutshell, to the best of our knowledge, this is the first study to evaluate the effect of NS extract on the gene expression of GST and CAT. Over-expression of pro-apoptotic genes may down regulate the expression of GST and CAT gene. These results may contribute to the basic understanding of the molecular mechanisms of the GST and CAT expression by methanolic extract of NS. Further studies are required to clarify the role of NS on the expression of antioxidant enzymes.

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