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RESEARCH ARTICLE

Antigenotoxic and antioxidant potentials of newly derivatized compound naringenin-oxime relative to naringenin on human mononuclear cells

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Abstract

We investigated antigenotoxic and antioxidative effects of newly derivatized compound naringenin-oxime (NG-Ox) compared to its mother compound naringenin (NG) against oxidative damage induced by hydrogen peroxide (HP) in human peripheral blood mononuclear cells (PBMC). Antigenotoxic activity was assessed using alkaline single cell gel electrophoresis assay (comet assay). Oxidative status was evaluated by measurement of total antioxidant status, total oxidant status and lipid hydroperoxide levels in the cells. Oxidative stress index was also calculated. Both NG and NG-Ox show a protective effect against HP-induced oxidative damage on PBMC and are able to reduce oxidative stress. The percentage of antigenotoxic and antioxidant potential progressively increased in a dose-dependent manner. However, these activities were found to be more significant in NG-Ox-treated cells than in NG-treated cells. Taken together, these observations provide evidences indicating that both NG and NG-Ox are able to protect cells against oxidative damage and apparently NG-Ox is more effective than NG.

Keywords

Comet assay, naringenin, naringenin oxime, natural antioxidant, total antioxidant status, total oxidative status

History

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Introduction

In living cells, when the formation of intracellular reactive oxygen species (ROS) exceeds the cell's antioxidant capacity, oxidative stress can arise, resulting in damage to cellular macromolecules such as proteins, lipids and DNA (Halliwell, 1991; Halliwell & Gutteridge, 1992; Sailaja Rao et al., 2011). DNA is a particularly sensitive cellular target because of the potential to create cumulative mutations that can disrupt cellular homeostasis (Hazra et al., 2007). DNA damage has also been considered as an important promoter of cancer, besides being implicated in the normal process of aging (Bjelland & Seeberg, 2003). Although cells have specific enzymatic and non-enzymatic mechanisms to neutralize ROS, these mechanisms cannot be enough to avoid oxidative modifications in lipids, proteins and DNA (Giorgio et al., 2007). Thus, attention has been focused on searching for natural products or their derivatives that can protect cells from oxidative damage (Lee & Kim, 2006). Flavonoids of plant origin have received increasing attention, especially in the field of pharmaceutical sciences and medicine, due to their potentials to prevent a number of chronic and degenerative diseases including cancer and cardiovascular diseases (Aluko et al., 2013; Bokkenheuser et al., 1991; Majo et al., 2005). These compounds can directly scavenge pathological concentrations of free radicals, chelate transition metal ions or interact with cellular signaling pathways involved in neurodegeneration (Ebrahimi & Schluesener, 2012).

Naringenin (NG), present in citrus and grapefruit, is a member of the flavonoid family (flavanone) that is considered to have various bioactivities on human health as antioxidant, ROS scavenger, monoamine oxidase inhibitor and especially cancer preventive agent (Liu et al., 1992; Olsen et al., 2008). It has been found that NG has antiproliferative, antiatherogenic, apoptotic and neuroprotective effects (Arul & Subramanian, 2013; Chanet et al., 2012; Park et al., 2008; Raza et al., 2013). NG has recently been used as a starting material for the synthesis of other novel derivatives. Özyürek et al. (2014) and Turkkan et al. (2012) synthesized, characterized and investigated the antioxidant properties of a new NG-derivatized compound called NG-Oxime (NG-Ox) and demonstrated that antioxidant properties of NG were significantly enhanced in its Ox form (Figure 1).

In this study, we assessed and compared, for the first time, the antigenotoxic and antioxidative potentials of NG-Ox relative to efficacy of NG against hydrogen peroxide (HP)-induced oxidative damage in human peripheral blood mononuclear cells (PBMC) *in vitro*.

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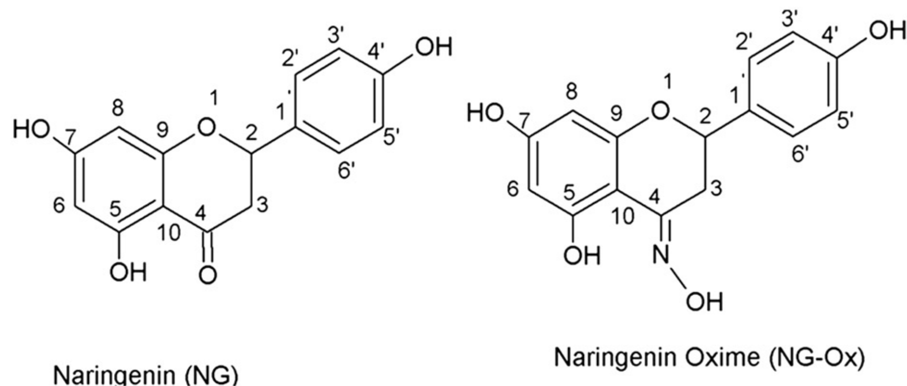

Figure 1. Chemical structure of NG and its new derivative NG-Ox.

Materials and methods

Chemicals

Naringenin ((±)-2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), heat-inactivated fetal calf serum, Dulbecco's modified Eagle medium (DMEM), Histopaque 1077 and NG were purchased from Sigma-Aldrich (Seelze, Germany). NG-Ox ((±)-2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one oxime) was synthesized and characterized by Turkkan et al. (2012). NG CAS Number: 67604-48-2, #W530098 Aldrich; hydroxylamine hydrochloride: CAS Number 5470-11-1 #159417, Sigma-Aldrich; sodium acetate trihydrate: CAS Number 6131-90-4, #S8625, Sigma-Aldrich. All other reagents used were of analytical grade unless otherwise stated.

Samples

Both NG and NG-Ox were dissolved in dimethyl sulfoxide (DMSO) to prepare 1280 μM stock solutions. The stock solutions were diluted with DMEM (containing no fetal bovine serum, FBS) for further use *in vitro*. The final concentration of DMSO in the NG and NG-Ox solutions were kept <1%. Prior to experiments, we confirmed that this level of DMSO as well as the serum free media did not induce any DNA damages in the cells. Other reagents were prepared as fresh before each experiment.

Isolation of human PBMC

Human peripheral blood was obtained by vein puncture from a non-smoker, healthy 23-year-old male volunteer. He was informed about the experimental procedures, signed consent form to participate in the study, and all the procedures were approved by the Ethics Committee of the Bezmialem Vakif University in keeping with the principles enunciated in the Declaration of Helsinki (Ethics Committee Decision Number: 71306642/050-01-04/287). He was not taking any type of medication. The PBMC isolation was made by the difference of gradient density (Hypaque 1077). After centrifugation (400*g*; 30 min at room temperature), the PBMC were found at the plasma/1077 interphase and collected carefully with a Pasteur pipette. After that, the cells were washed in PBS twice (240*g* for 10 min), and re-suspended in DMEM medium containing 4.5 g/L glucose supplemented with 2 mM L-glutamine, penicillin/streptomycin (50 IU/mL and 50_g/mL, respectively) and 10% (v/v) FBS. Viability of the cells was evaluated by trypan blue dye exclusion method and the range of viable cells was within 90% prior to cultivation. All cells were cultured in DMEM equilibrated with 5% CO_2 atmosphere at 37 °C. The cell culture was maintained in 6-well and 96-well cell culture plates.

Cell viability assay

The cytotoxicities of NG and NG-Ox were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay which is based on the cleavage of tetrazolium salts by mitochondrial succinate-tetrazolium reductase in viable cells to form formazan dye (Mosmann, 1983). PBMC were seeded in 96-well plates at a density of 5×10^3 cells/well and allowed to attach. After 24 h exposure to the NG and NG-Ox at different concentrations (20–1280 μM), the medium was then removed and the cells were slightly washed with PBS. MTT (5 mg/mL) was added, and after 3 h of incubation at 37 °C the resulting formazan crystals were dissolved in DMSO. Cell viability was measured in terms of absorbance at 570 nm in a microplate reader (Varioskan Flash Multimode Reader, Thermo, Waltham, MA) and, the reduction of cell viability was expressed as the percentage compared with control group designated as 100%. Three individual wells were measured per treatment point.

Preparation of the cells for antigenotoxic and anti-oxidative assays

To determine the antigenotoxic potential of the both NG and NG-Ox against HP-induced oxidative DNA damage, PBMC were seeded onto 6-well plates (approximately 1×10^5 cells per well) with cell culture medium and incubated at 37 °C in 5% CO_2 for 24 h. The cells were then pre-treated with NG and NG-Ox at non-cytotoxic concentrations of 20–340 μM in the well for another 24 h. DMSO (1%) was used as a negative control. After pre-treatment, the cells were exposed to 50 μM of HP (final concentration in the cell suspension) for 5 min on ice to induce oxidative damage. The cells were then harvested using a cell scraper, centrifuged and re-suspended in 1 mL of PBS. Ten microliters of re-suspended cells were placed into Eppendorf tubes for related measurements of the comet assay as described below.

To determine of endogenous oxidative status, remaining cells were centrifuged at 800*g* (4 °C) for 5 min and the supernatant was discarded. The pellets were then re-suspended in cell lysate buffer [20 mM HEPES (pH 7.4) containing 0.5% Triton-X 100, 1 mM EDTA]. The suspended cells were lysed by vortexing and incubating on ice for 20 min. The cell lysate was centrifuged for 5 min at 14 000*g* (4 °C) and resulting supernatant was preserved to measure the levels of lipid hydorperoxide (LOOH), total antioxidant status (TAS) and total oxidant status (TOS). Protein concentration of the lysate was determined against a commercially available protein standard (Bio Rad, Hercules, CA) using Bradford protein assay (Bio Rad detergent compatible assay reagent, Bio Rad) and BSA as the standard. All manipulations were performed at 4 °C.

DNA damage assay

The alkaline single-cell gel electrophoresis assay (comet assay) was used to investigate the potential protective effects of the test compounds against DNA damage in PBMC stressed with HP. Comet assay was conducted according to Singh et al. (1988) with slight modifications (Kocyigit et al., 2011) as follow: 10 μL cell suspension (see above) was mixed with 75 μL of 0.6% low melting agarose (LMA) and was immediately covered with a coverslip. Slides were left for 5 min at 4 °C to allow the agarose to solidify. After the coverslips were removed, the slides were submersed in freshly prepared cold (4 °C) lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, pH 10–10.5, 1% Triton X-100 and 10% DMSO added just before use) for at least 1 h. LMA and immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO, pH 10) for 1 h at 4 °C. After the slides were removed from lysis solution, they were washed with cold PBS and placed in an electrophoresis tank horizontally side by side. DNA was allowed to unwind for 30 min in freshly prepared alkaline electrophoresis buffer containing 300 mM NaOH and 10 mM Na_2EDTA (pH 13.0). After unwinding, electrophoresis was run at 300 mA for 25 min at 4 °C under minimal illumination to prevent further DNA damage. The slides were washed three times with a neutralization buffer (0.4 M Tris, pH 7.5) for 5 min at 4 °C and then treated with ethanol for another 5 min before staining. Dried microscope slides were stained with ethidium bromide (2 μg/mL in distilled H_2O; 70 μL/slide) covered with a coverslip and analyzed using a fluorescence microscope (Leica DM 1000, Solms, Germany) at a 200 × magnification with epiflourescence equipped with a rhodamine filter (with an excitation wavelength of 546 nm; and a barrier of 580 nm). A hundred cells were randomly scored by eye in each sample on a scale of 0–4 based on fluorescence beyond the nucleus. The scale used was as follows: 0, no tail; 1, comet tail < half the width of the nucleus; 2, comet tail equal to the width of the nucleus; 3, comet tail longer than the nucleus; 4, and comet > twice the width of the nucleus. Scoring cells in this manner have been shown to be as accurate and precise as using computerized image analysis. The individual scoring of the slides was blind, using coded slides. The visual score for each class was calculated by multiplying the percentage of cells in the appropriate comet class by the value of the class. The total visual comet score characterizing the degree of DNA damage in the entire study groups was the sum of the scores in the five comet classes. Thus, the total visual score could range from 0 (all undamaged) to 400 (all maximally damaged) arbitrary units (AU), as reported by Collins et al. (1995). This method of measurement was proved to be valid and up-to-date (Dikilitas & Kocyigit, 2012). All of the procedures were completed with the same biochemistry staff and DNA damage was detected using a single observer that was not aware of the subject's status. Cell viability measured with trypan blue exclusion test was above 95% for all treatments. Results of the triplicated tests are expressed in terms of AU recorded. All experiments were repeated in triplicate.

Determination of intracellular TAS

Intracellular cell TAS level was measured using a novel automated colorimetric measurement method developed by Erel (2004). In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate *O*-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the lysate, preventing the color change and thereby providing an effective measure of the TAS of the lysate. The assay results are expressed as mmol Trolox equiv/mg protein.

Determination of intracellular TOS

Intracellular TOS (Sakihama et al., 2000) level was measured using an automated colorimetric measurement method developed by Erel (2005). In this method, oxidants present in the sample oxidize the ferrous ion-*O*-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules (e.g. lipids, proteins) present in the sample. The assay is calibrated with HP, and the results are expressed in terms of micromolar hydrogen peroxide per mg protein (μmol HP/mg protein).

The percent ratio of TOS to TAS level has been accepted as the oxidative stress index (OSI) (Dikilitas et al., 2011; Erel, 2004).

OSI value was calculated according to the following formula:

$$\mathrm{OSI} = \frac{\mathrm{TOS},\ \mu\mathrm{mol\ HP\ equiv/g}}{\mathrm{TAS},\ \mu\ \mathrm{mol\ Trolox\ equiv/g}} \times 10.$$

Determination of Intracellular LOOH

LOOH concentrations of cell lysate were determined using the FOX2 method with minor modifications (Harma et al., 2003). The FOX2 test is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides in the plasma samples, in the presence of xylenol orange, which produces a

colored ferric–xylenol orange complex whose absorbance can be measured. The color intensity is related to the total amount of lipid oxidations. The LOOH content of the lysate was determined as a function of the difference in absorbance between the test and blank samples using a solution of HP as the standard. The coefficient of variation for individual lysate samples was less than 5%.

Statistical analysis

All outcomes including the measured concentrations of proteins, LOOH, TAS and TOS were evaluated statistically and the final results were expressed as mean ± SD. The results are presented as three replicates. Data in the experiments were analyzed using analyses of variance (one-way ANOVA). Associations between antigenotoxic activity and oxidative stress parameters were analyzed by Pearson correlation coefficient. The p value < 0.05 was considered as statistically significant. All statistical analyses were performed using SPSS package program for Windows (Version 11.5, Chicago, IL).

Results

The effects of NG and NG-Ox on inhibition of growth and proliferation of PBMC were determined via MTT reduction assay (Figure 2). While NG significantly inhibits PBMC proliferation at the concentration of 1280 µM, NG-Ox inhibits the cell proliferation at the concentrations of 640 and 1280 µM. Non-cytotoxic concentrations were further used in DNA damage and investigations caused by these two compounds.

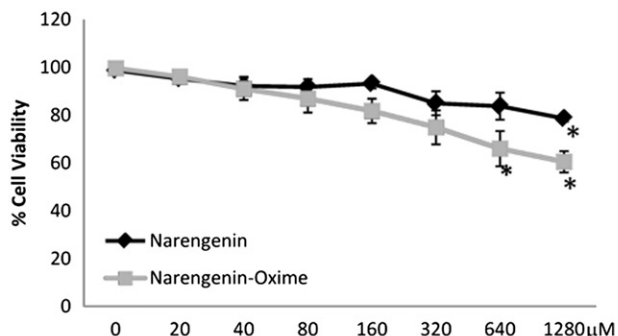

Figure 2. Effect of NG-Ox and NG on the mononuclear leukocyte cell viability for 24 h. * indicates active concentrations. Data are expressed as the mean ± standard deviation of three independent experiments.

The protective effect of the NG and NG-Ox on PBMC cells from HP-induced DNA damage was investigated using the comet assay. In the set, PBMC pre-treated with NG and NG-Ox at non-cytotoxic concentrations (20–320 µM) showed a significant increase in DNA protection ($p < 0.05$). The antigenotoxic effects of NG and NG-Ox against HP-induced oxidative damage in PBMC are presented in Figure 3. Theantigenotoxic activity progressively increased in a dose-dependent manner and, the highest antigenotoxic effect was determined from 49 to 23 AU for Ng-Ox (53%) and, 48.6 to 33 AU for NG (32%) at a concentration of 320 µM induced by HP (50 µM).

PBMC incubated with HP alone (as a positive control) exhibited a significant decrease in TAS levels when compared to the negative control. However, treating with NG and NG-Ox prior to HP caused an increase in the intracellular TAS levels compared to the positive control (Figure 4). The highest TAS level was achieved by pre-treating cells with 320 µM of NG-Ox. The dominant efficacy of 160 µM NG-Ox in increasing the TAS value compared to that of NG in the same concentration is notable.

The TOS level of the cells, incubated with NG and NG-Ox prior to treatment with HP, significantly decreased compared to those treated only with positive control (HP). However, TOS level of NG-Ox-treated cells was significantly lower than those of NG-treated cells at the concentration of 80 µM (Figure 5).

Since OSI is an index of oxidative stress obtained by dividing TOS to TAS (µmol HP equivalent/µmol Trolox equiv), there is no certain unit to express the OSI results, thus these results are being reported as AU. The results are presented in Figure 6. As seen in the figure, both NG and NG-Ox treatment in all doses decreased the intracellular OSI levels induced by HP. NG-Ox at the concentrations of 40 and 160 µM was predominantly active in decreasing the OSI levels compared to NG at the same concentrations.

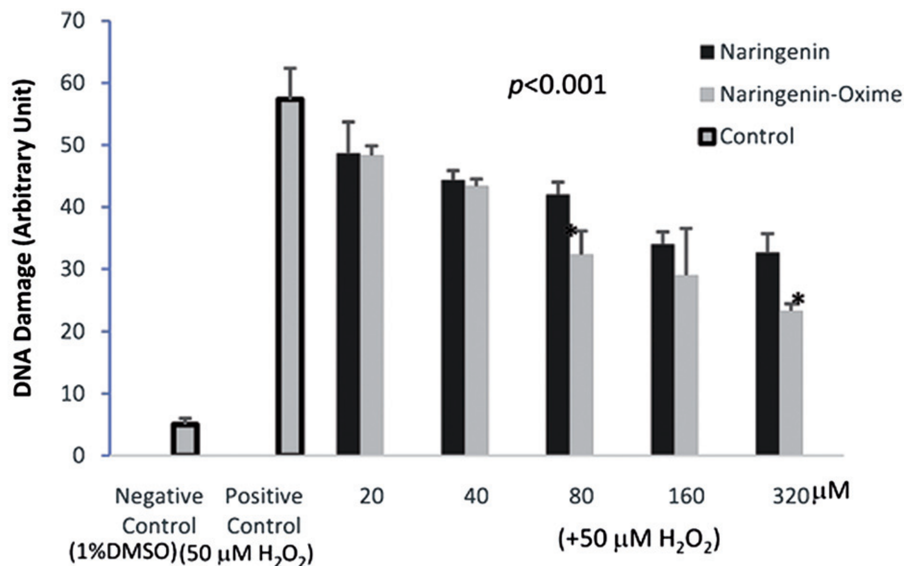

Figure 3. Prevention of H_2O_2-induced genotoxicity in PBMC treated with various concentrations of NG and NG-Ox. DNA damage was determined using the comet assay. Data are expressed as the mean ± standard deviation of three independent experiments. Values marked with * indicate significant differences between NG and NG-Ox at the same dose of treatment.

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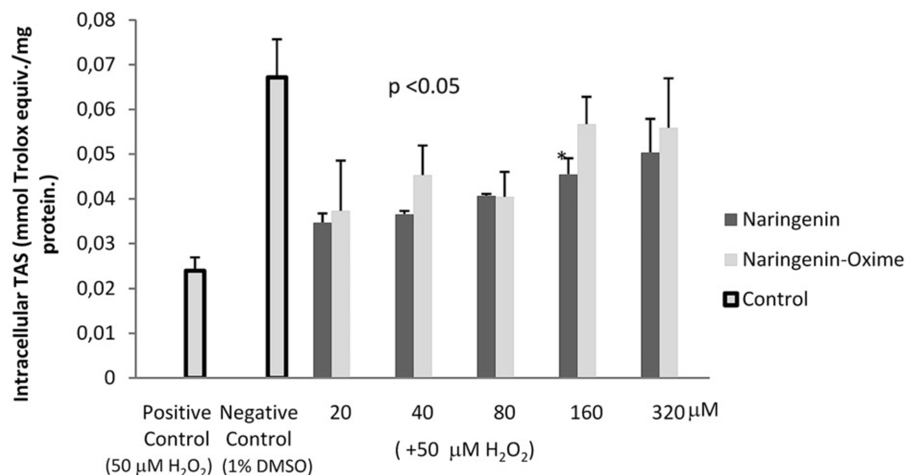

Figure 4. Effects of NG-Ox and NG on intracellular TAS induced by H_2O_2 (50 μM) in human PBMC. Data are expressed as the mean ± standard deviation of three independent experiments. Values marked with * indicate significant differences between NG and NG-Ox at the same dose of treatment.

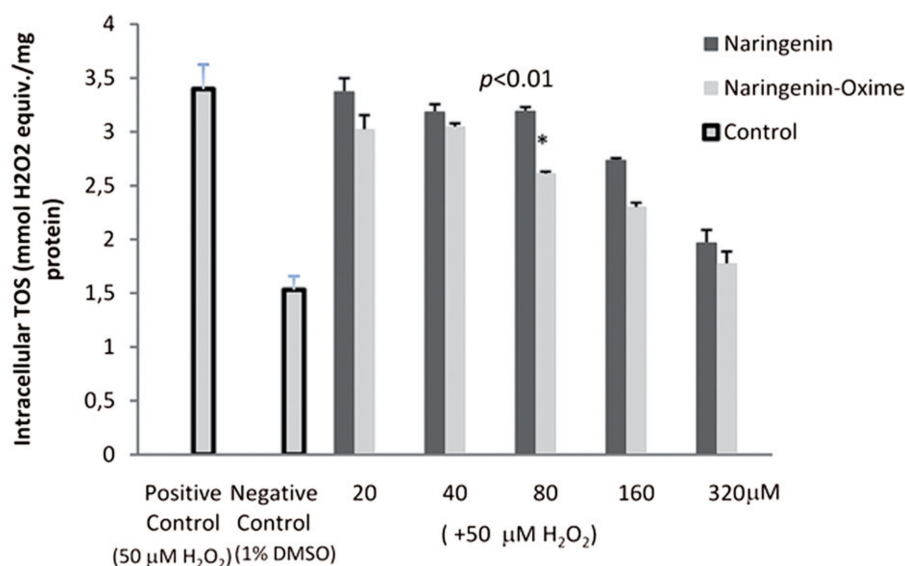

Figure 5. Effects of NG-Ox and NG on intracellular TOS induced by H_2O_2 (50 μM) in human PBMC. Data are expressed as the mean ± standard deviation of three independent experiments. Values marked with * indicate significant differences between NG and NG-Ox at the same dose of treatment.

Compared with the positive control (50 μM HP alone), LOOH level of NG- and NG-Ox-treated cell was found significantly lower following the treatment of HP (Figure 7). The level of LOOH in NG-Ox-treated cells was found significantly lower than those of treated cells with NG at the concentrations of 20, 40 and 80 μM.

A close relationship existed between the DNA damage and OSI levels in NG and NG-Ox treated cells ($r = 0.966$, $p < 0.001$ and $r = 0.936$, $p < 0.01$, respectively) against oxidative stress using HP.

Discussion

Oxidative agents such as HP, which is the main source of ROS, have an important role in the pathogenesis of a wide range of diseases including cancers (Kaur et al., 2009; Kumar & Chattopadhyay, 2007). The damage caused by free radicals can be prevented by natural or synthetic scavengers. Epidemiological data showed that the plant extracts could prevent the diseases such as cancer and other chronic diseases. Quite a few studies have demonstrated that some flavonoids possess several biological and pharmaceutical activities including anticancer, antioxidative and antigenotoxic effects (Liu et al., 2010). Therefore, dietary flavonoids or their derivatives have attracted a great attention as antigenotoxic and chemopreventive agents. A new NG-derivatized compound, NG-Ox, has been demonstrated antioxidative and cytotoxic properties by Turkkan et al. (2012) and Özyürek et al. (2014). However, there is no report available investigating the antigenotoxic and antioxidative effects of this new derivative compound on eukaryotic normal cells. We, therefore, investigated for the first time the antigenotoxic and antioxidant effects of NG-Ox relative to efficacy of free NG against oxidative DNA damage induced by HP.

The possible antiproliferative effects of the NG-Ox and NG were evaluated using MTT assay. Although we were not able to calculate the IC_{50} values (Figure 2), the concentration of NG-Ox up to 640 μM did not affect the viability of PBMC cells after 24 h of exposure but above this concentration, NG-Ox started to be toxic and the cell viability dropped under 60.5%. This is where NG inhibits PBMC proliferation solely at the concentration of 1280 μM. Several *in vitro* studies also demonstrated that NG has antiproliferative effects in cancer cell lines as well and the literature findings (Kanno et al., 2006; Park et al., 2008) are in agreement with our results obtained with NG. However, there is no report about NG-Ox. Flavonoids are universally recognized antioxidants which can

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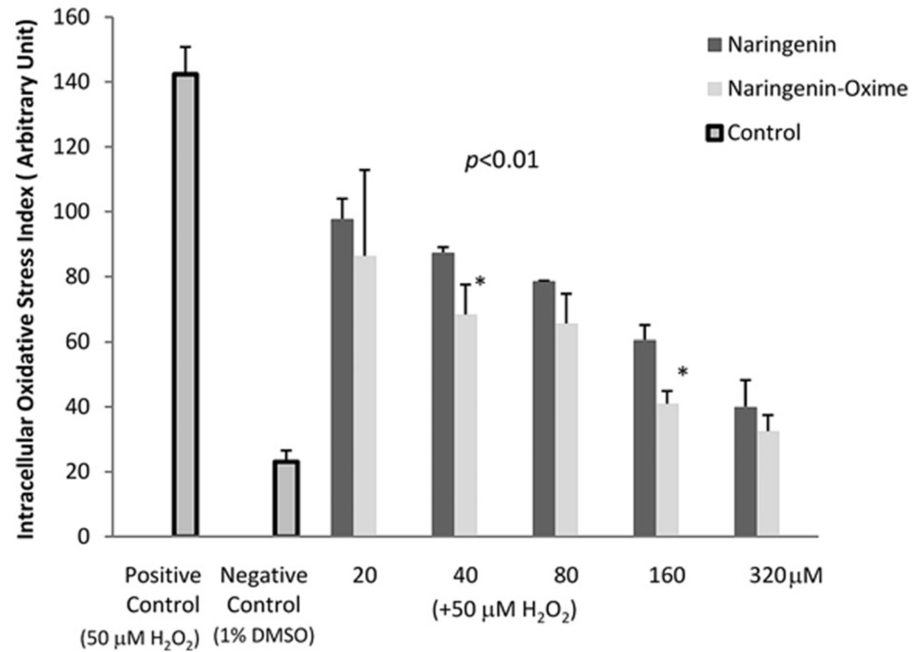

Figure 6. Effects of NG-Ox and NG on intracellular OSI level induced by H_2O_2 (50 μM) in human PBMC. Data are expressed as the mean ± standard deviation of three independent experiments. Values marked with * indicate significant differences between NG and NG-Ox at the same dose of treatment.

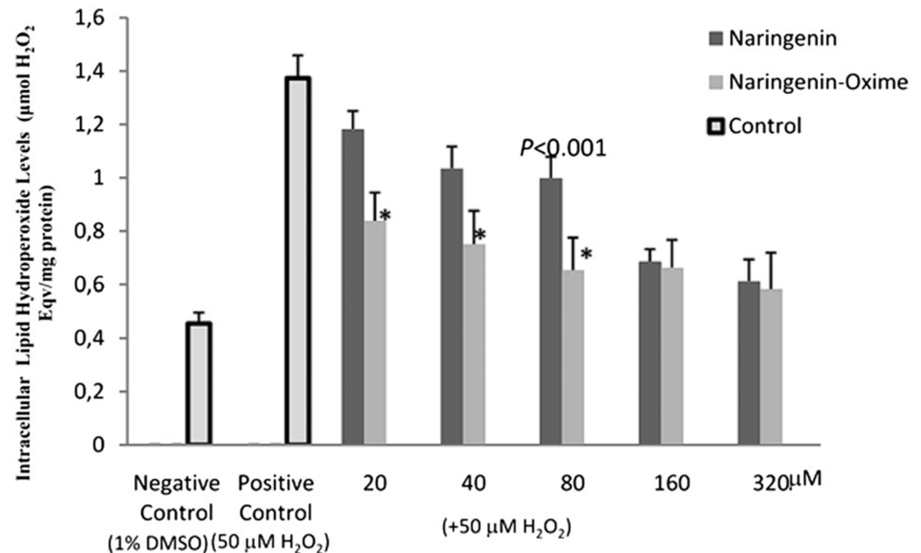

Figure 7. Effects of NG-Ox and NG on intracellular lipid hydroperoxide level induced by H_2O_2 (50 μM) in human PBMC. Data are expressed as the mean ± standard deviation of three independent experiments. Values marked with * indicate significant differences between NG and NG-Ox at the same dose of treatment.

protect the cell from the oxidative stress, i.e. neutralize the damaging effect of ROS. However, this favorable property of flavonoids comes along with their unwanted cytotoxic activity at their high concentrations (Maroziene et al., 2012). The mechanism(s) of the selective inhibitory effect on cell growth has not been elucidated yet. However, it was demonstrated that NG had a pro-oxidant potential (Galati et al., 2002). Concurrently with previous studies NG showed antiproliferative (cytotoxic) effect above 640 μM, while NG-Ox was even more toxic in this concentration. Based on these results, we used the maximal concentration of 320 μM of examined compounds in which neither NG nor NG-Ox shows any genotoxic efficacy for further experiments.

We investigated the ability of NG-Ox and NG to protect PBMC against oxidative stress caused by HP as the free radical generating system. Although HP itself does not have the properties of a free radical, it is a potential source of the dangerous hydroxyl radical ($OH^{\cdot -}$) which could attack DNA leading to fragmentation, base loss and strand breaks (Diplock, 1991). In the present study, due to induction of oxidative stress, HP produces various levels of damage to the DNA of the isolated PBMC. In this study, 50 μM HP was chosen to induce DNA damage, as this dose was not cytotoxic (cell viability > 90%). To assess the possible antigenotoxic action of NG-Ox and NG, we monitored the comet assay which is one of the standard methods for assessing DNA damages including single- and double-strand DNA breaks (Behravan et al., 2011; Kassie et al., 2000). It assesses the effectiveness of compounds to protect cells from genotoxic damage. In the present study, both NG-Ox and NG were found to have a striking ability to reduce the DNA-damaging effect of HP; however, NG-Ox was found to have a higher activity (Figure 3). In agreement with our result, Çelik & Koşar (2012) have also demonstrated the antigenotoxic effect

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of NG against idarubicin-induced DNA damage. According to our knowledge this study is the first report on the antigenotoxic activity of NG-Ox and NG.

It has been demonstrated that, in general, there is a close relationship between antigenotoxic activity and antioxidative potentials of many plants (Zegura et al., 2011). However, the precise cellular and molecular mechanisms underlying such effects of flavonoids such as NG are largely unknown. Several mechanisms have been proposed for their antigenotoxic effects. In simultaneous incubation experiments, these compounds may protect DNA against oxidant-induced damage directly, either by free radical scavenging activity or by decreasing free radical production (Anderson et al., 2000). Flavonoids composed of one or more aromatic rings bearing one or more hydroxyl groups, can readily combine with free radicals forming resonance-stabilized phenoxyl radicals (Rice-Evans et al., 1996). Several studies suggest that the multiple hydroxyl groups and catechol rings are the key factors in their antioxidative scavenging actions (Ordoudi & Tsimidou, 2006). Their antioxidant action resides mainly in their ability to donate electrons or hydrogen atoms (Arora et al., 1998; Sakihama et al., 2000). As seen in Figure 5, while NG has three OH groups, NG-Ox has four of them. The oxime-compound is possibly working by providing hydrogen atoms from their phenolic hydroxyl groups to scavenge hydroxyl radicals generated by HP resulting in enhancement of antigenotoxic activity.

A number of methods are available for assessing antioxidant activity, and these methods vary substantially in terms of complexity, chemistry and other factors. Concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive and costly. As the effects of the antioxidative components in the samples are additive, measurement of the total antioxidant response accurately reflects the redox status of the sample (Halliwell, 1994). Thus, instead of measuring individual antioxidant components of sample as single tests, the TAS may be more useful and practical for evaluating the antioxidant status of samples (Harma et al., 2005). In the present study, TAS levels in NG-Ox- and NG-treated PBMC were significantly higher against the oxidative damage produced by HP and, NG-Ox was more active than in NG. Our results are also in good agreement with Özyürek et al. (2014) and Turkkan et al. (2012).

Also there are various methods to assess the oxidative damage of cells (Holley & Cheeseman, 1993), but a direct estimation of the ROS provides a good indication of the oxidative damage (Reddy et al., 2008). TOS and OSI are accepted cumulative markers for oxidative power (Aycicek & Ipek, 2008). In this study, we showed the significant increase of TOS and OSI levels in cells treated with HP as well as its significant suppression in the presence of NG and NG-Ox, while NG-Ox is being the more effective one. The oxidative stress suppression of NG-Ox may be is in coordination and dependent to its antioxidative potential.

An important step in the degradation of cell membrane is the reaction of ROS with the double bonds of polyunsaturated fatty acids to yield lipid hydroperoxides. On breakdown of such hydroperoxides, a great variety of aldehydes are formed (Esterbauer et al., 1991). Given the limitations of the indirect methods, we used the direct measurement of LOOH (Yamamoto et al., 1987). By using a direct and more sensitive method for LOOH, we have demonstrated an increase of LOOH concentration in PBMC treated with HP alone, indicating a significant degree of lipid peroxidation. In our experimental conditions, the decrease of LOOH levels was observed in the cells co-treated with NG, NG-Ox which suggests significant protection against the lipid peroxidation due to the effectiveness of these compounds again with the higher effectiveness obtained from co-treatment of NG-Ox.

Our results suggest that the use of these two compounds may afford a cytoprotective effect due to their significant inhibiting potential of lipid peroxidation. The protective action of the tested compounds can be explained by their ability to penetrate through the cell membrane and to interrupt the radical chain induced by HP, thus, allowing the prevention or reduction of free radical formation, which are responsible for cellular macromolecules damage (Valko et al., 2007). Similarly, $DPPH^+$ free radical scavenging and lipid peroxidation inhibition activity of the essential oil fraction of a related species (*Cymbopogon citratus*) on human erythrocytes have been reported earlier (Cheel et al., 2005). We also suggest that NG-Ox may have more free radical scavenging activity compared to NG because of its chemical structure.

Conclusion

In conclusion, the present investigation demonstrated that newly synthesized oxim derivative of NG, resulted in more antigenotoxic and antioxidative potential than the mother compound NG. Although, additional research is needed to delineate the relative contribution of this pathway to antigenotoxic and antioxidative activity, these findings could be used to design cancer preventive flavanone-derived agents.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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