Araştırma Makalesi / Research Article

Anti-genotoxic and anti-cytotoxic potential of black cumin seed (*Nigella sativa*) extract on aluminum-exposed human lymphocyte cells

Alüminyum toksisitesine maruz bırakılmış insan lenfosit hücrelerinde çörek otu (*Nigella sativa*) ekstraktinin anti-genotoksik ve anti-sitotoksik etkileri

Hakim ÇELİK[1], Cahit BAĞCI[2], Abdurrahim KOÇYİĞİT[3], İsmail KOYUNCU[4], Ali Ziya KARAKILÇIK[1]

[1] Harran Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, Şanlıurfa, Türkiye
[2] Sakarya Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, Sakarya, Türkiye
[3] Bezmialem Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, İstanbul, Türkiye
[4] Harran Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Şanlıurfa, Türkiye

Abstract

Background: The third most common element in the world, aluminum (Al), causes genotoxicity, cytotoxicity, and oxidative stress when its accumulation increases in the body. Conversely, *Nigella sativa* seeds (NSS) are a unique source of potent antioxidants, such as thymoquinone (TQ) and phenolic compounds. It is currently unclear if NSS have protective potential against the toxic efficiencies of Al. Therefore, this study aimed to assess the anti-genotoxic and anti-cytotoxic effects of the methanolic extract of *Nigella sativa* seeds (NSE) at different concentrations in human peripheral blood lymphocyte cells (PBLC) against Al toxicity in *in vitro* conditions.

Material and Methods: A comet assay, micronucleus test, DNA fragmentation analyses, and methyl-thiazol-tetrazolium (MTT) assay were used to evaluate the anti-genotoxic, anti-cytotoxic, and anti-clastogenic effects of NSE against the Al toxicity.

Results: The obtained data showed that Al had genotoxic and cytotoxic effects at 100 µM of concentration ($p<0.05$) and that NSE at low concentrations (0.1–2 µg/mL) had anti-genotoxic and anti-clastogenic effects against Al-toxicity ($p<0.05$). It was also observed that NSE at or above 2 µg/mL of concentration had genotoxic effects and that NSE at or above 5 µg/mL of concentration had cytotoxic effects ($p<0.05$).

Conclusions: This study recommends that the use of materials made of Al should be restricted and that NSS should be consumed in low quantities to neutralize Al toxicity.

Keywords: Aluminum toxicity, Nigella sativa, Genotoxicity, Cytotoxicity.

Öz.

Giriş: Dünyadaki en yaygın üçüncü element olan alüminyum, vücutta biriktiğinde genotoksisite, sitotoksisite ve oksidatif strese neden olmaktadır. Çörek otu ise timokinon ve fenolik bileşikler gibi güçlü antioksidanların kaynağı olduğu bilinmektedir. Ancak çörek otunun, alüminyum toksisitesine karşı koruyucu etkiye sahip olup olmadığı henüz araştırılmamıştır. Bu nedenle, çalışmamızda alüminyum toksisitesine karşı çörek otunun anti-genotoksik ve anti-sitotoksik etkilerini in vitro hücre kültürü ortamında araştırmayı amaçladık.

Materyal ve Metod: İnsan lenfosit hücrelerinde, alüminyum toksisitesine karşı, çörek otunun antisitotoksik ve antigenotoksik etkileri, alkali tek hücre elektroforez yöntemi (comet assay), mikronükleus test, metil tiyazol tetrazolium (MTT) test ve DNA fragmantasyon analizi ile çalışıldı.

Bulgular: Elde edilen bulgular, alüminyumun 100 µM'da genotoksik ve sitotoksik etkili olduğunu ($p<0,05$) ve düşük konsantrasyonlarda (0,1-2 µg / mL) çörek otu ekstraktının alüminyum toksisitesine karşı anti-genotoksik ve anti-klastojenik etkilere sahip olduğunu göstermektedir ($p<0,05$).

Sonuç: Bu çalışma, genotoksik ve sitotoksik etkilerinden dolayı alüminyumdan yapılmış malzemelerin kullanımının kısıtlanması ve alüminyum toksisitesini nötralize etmek için düşük miktarlarda çörek otunun tüketilmesi gerektiğini önermektedir.

Anahtar Kelimeler: Alüminyum toksisitesi, Çörek otu, Genotoksisite, Sitotoksisite.

Sorumlu Yazar / Corresponding Author

Dr. Hakim Çelik

Harran Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, Şanlıurfa, Türkiye

Tel: +90 532 762 45 83

E-mail: hakimcell@gmail.com

Geliş tarihi / Received: 09.10.2018

Kabul tarihi / Accepted: 01.11.2018

Introduction

Aluminum (Al), which is one of the most abundant elements found in the Earth's crust, is an unnecessary element that is toxic to living organisms (1,2). As Al is broadly dispersed in the environment and used widely in daily life, humans are often exposed to it (3–5). The human body obtains Al through occupational exposure and from cosmetics, toothpaste, inhaled fumes, drinking water, processed foods, antiperspirants, and adjuvants in various pharmaceutical agents and parenteral preparations (1,2,4–6). The increasing use of Al cans, foils, and vessels in the preparation and storage of food increases this intake; the acidity, saltiness, or increased alkalinity of food increases it further (2,7). Al is known to accumulate in all human tissues and to be toxic to various organ systems, including the lung, bone, brain, liver, heart, muscle, kidneys, and blood cells (1,4,5,8).

While Al is a redox-inactive metal, it is thought to have strong pro-oxidant activity (1,2,9). It modulates the ability of pro-oxidant metals (such as iron and copper) to boost metal-based oxidative stress (OS), and it causes oxidative damage by binding to them (1,10,11). There is strong evidence that Al forms the Al superoxide anion complex, which is a more potent oxidant than superoxide anion on its own, as this superoxide anion complex promotes the formation of hydrogen peroxide and hydroxyl radicals, which generate OS (1,9). It is also known that increased OS can lead to DNA damage, rising sensitivity to apoptosis, which can result in mutagenic, cytotoxic, or carcinogenic events, and the attenuation of DNA repair mechanisms (1,11,12). Studies have indicated that medicinal plants with antioxidant and/or free radical scavenging properties should be used to mitigate metals that induce intoxication.

In many countries in Western Asia, Eastern Europe, the Middle East, and the Mediterranean, black cumin (*Nigella sativa L.*), which belongs to the botanical family of Ranunculaceae, has long been used in folk medicine and in the flavoring of breads and pickles (13). *Nigella sativa* seeds (NSS) have many important active ingredients that exhibit strong antioxidant and free radical scavenging properties, such as thymol, carvacrol, quercetin, nigellicine, nigellidine, thymoquinone (TQ) (30–48%), dithymoquinone, thymohydroquinone, dithymoquinone, and nigellimine N-oxide (14–16). Previous studies have identified the many medical properties of NSS, which include immune stimulation and anti-cancer, antioxidant, antidiabetic, anti-microbial, and anti-inflammatory effects (15,17–19). It has been reported that NSS, and TQ as their active ingredient, inhibit lipid peroxidation in the membranes of tissues (20). It has also been indicated that their free-radical scavenging capability is as effective as that of superoxide dismutase (21,22).

As mentioned above, several studies have demonstrated the cytotoxic and genotoxic effects of Al. However, no study has investigated the efficacy of the methanolic extract of *Nigella sativa* seeds (NSE) against the genotoxic and cytotoxic effects of Al in human peripheral blood lymphocyte cells (PBLC). For this reason, the current study aimed to investigate the anti-genotoxic and anti-cytotoxic effects of NSE against the cytotoxic and genotoxic effects of Al in *in vitro* conditions.

Material and Methods

Chemical

RPMI 1640 Medium, Histopaque 1077, aluminum chloride (AlCl3), and phytohemagglutinin (PHA-M) were obtained from Sigma-Aldrich (Seelze, Germany). In addition, all other reagents that were used in this study were of analytical grade.

Preparation of AlCl3 Solution

A stock solution of 500 mM of AlCl3 was prepared in distilled water. The stock AlCl3 solution was diluted with RPMI 1640 Medium in the processing of the experimental analyses.

Preparation of NSE

The NSS were purchased from a local herbalist in Sanliurfa, Turkey. After they were botanically identified and authenticated, the seeds (100 g) were washed, dried, and crushed into a fine powder with an electric grinder (Delonghi KG79, Italy) and then extracted with 70% aqueous methanol (1: 4, w/v) for 24 hours. The NSE was percolated through a filter paper (Whatman Grade 3, Sigma-Aldrich, Germany) and concentrated using a vacuum rotary evaporator (Buchi R-200, Switzerland). The concentrated NSE was frozen at -80 °C and freeze-dried in a Freeze-dryer chamber (ilShin Lab Co. Ltd., South Korea). The dark brown concentrated NSE was weighed and stored in a dark glass vial at -80 °C until use. The NSE was first dissolved in dimethyl sulfoxide (DMSO) to prepare 1 mg/mL of stock solution, and then the stock solution was diluted with RPMI 1640 Medium for later use. The ultimate DMSO concentration of the NSE solutions was made to be less than 1%. Before the experiments were conducted, it was verified that this level of DMSO did not cause any damage to the DNA within the PBLC.

Cell Culture and Experimental Design

Human peripheral blood was taken from a healthy, non-smoking, 37-year-old male volunteer who was not taking medication. He was informed about the study's procedure and signed a consent form to participate. The study's procedure was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Harran University.

A peripheral blood sample was collected in a heparinized tube after 8–12 hours of overnight fasting, and PBLC were freshly separated using Histopaque 1077. The PBLC were isolated from the entire blood sample using a density-gradient separation technique. After centrifugation (30 minutes at 400 × g, 20 °C), the PBLC were located at the interface between the plasma and Histopaque 1077 and carefully aspirated using a sterile Pasteur pipet. The harvested PBLC were washed twice in a phosphate-buffer solution (PBS) and centrifuged at 240 × g for 10 minutes. After that, the PBLC were suspended in a RPMI 1640 Medium that had been supplemented with 20% (v/v) Fetal bovine serum (FBS), 5 µg/mL of Gentamicin, and 5 µg/mL of PHA-M to prepare the PBLC-suspended solution (PBLC-SS). Using the trypan blue exclusion method to determine the viability of the cells prior to their cultivation, the viable cell ratio was found to be greater than 90%. 24-well and 96-well cell culture plates were used for cell cultivation. NSE and AlCl3 were added at the planned concentration, and the PBLC were incubated in a humidified CO2 incubator (5% CO2, 95% air) at 37°C for 72 hours. All experiments were repeated at least three times, and the study was performed in a preliminary studies stage and a main studies stage.

Preliminary Studies Stage

In this stage, we aimed to determine the effective doses of AlCl3 and NSE on PBLC at different concentrations. The comet assay method was used to determine the probable toxic concentration of AlCl3 and the possible protective concentration of NSE. Hence, for the different concentrations of AlCl3, the cell culture plate wells were classified into the following five groups: control (PBLC-SS), Al-25 (PBLC-SS + 25 µM of AlCl3), Al-50 (PBLC-SS + 50 µM of AlCl3), Al-100 (PBLC-SS + 100 µM of AlCl3), and H2O2-50 (PBLC-SS + 50 µM of H2O2). After identifying the suitable toxic concentration of AlCl3 (100 µM) that could be used in this study, the cell culture plate wells were classified into the following seven groups to determine the most effective anti-genotoxic concentration of NSE against the toxic effect of 100 µM of AlCl3: control (PBLC-SS), Al-100 (PBLC-SS + 100 µM of AlCl3), NSE-0.1 (PBLC-SS + 100 µM of AlCl3 + 0.1 µg/mL of NSE), NSE-1 (PBLC-SS + 100 µM of AlCl3 + 1µg/mL of NSE), NSE-2 (PBLC-SS + 100 µM of AlCl3 + 2 µg/mL of NSE), NSE-5 (PBLC-SS + 100 µM of AlCl3 + 5 µg/mL of NSE), and H2O2-50 (PBLC-SS + 50 µM of H2O2). As a result, the most effective anti-genotoxic concentration of NSE that this study could use against the toxic effect of 100 µM of AlCl3 was determined to be 1 µg/mL.

Main Studies Stage

After optimal doses of AlCl3 and NSE were determined by the preliminary studies, cell culture plate wells were divided into the following four groups to investigate their genotoxic and cytotoxic effects: control (PBLC-SS), Al-100 (PBLC-SS + 100 µM of AlCl3), NSE-1 (PBLC-SS + 100 µM of AlCl3 + 1 µg/mL of NSE), and H2O2-50 (PBLC-SS + 50 µM of H2O2).

Determination of DNA Damage by Comet Assay

An alkaline single-cell gel electrophoresis assay (comet assay) was performed to Singh et al.'s specifications (23) with the minor modifications (24) that follow: 10 µl of PBLC-SS (roughly 2 × 104 cells) were mixed with 80 µl of 0.7% low melting agarose (LMA) at 37 °C and were layered onto slides that had been precoated with a thin layer of 1% normal melting agarose (NMA). The slides were immediately covered with a coverslip and were left at 4 °C for 5 minutes to allow the agarose to solidify. After removal of the coverslips, the slides were immersed in cold (4 °C) and freshly prepared lysis solution (2.5 M of NaCl, 100 mM of Na2EDTA, 10 mM of Tris–HCl, pH 10–10.5; 1% Triton X-100 and 10% DMSO added just before use) for 1 hour. After the slides were removed from the lysis solution, they were washed with cold PBS and placed horizontally in an electrophoresis tank side by side. DNA was allowed to unwind for 30 minutes in freshly prepared alkaline electrophoresis buffer (300 mM of NaOH and 10 mM of Na2EDTA, pH 13.0) and then electrophoresed (25 V/300 mA, 25 minutes) under minimal illumination to prevent further DNA damage. The slides were washed three times with a neutralization buffer (0.4 M of Tris, pH 7.5) at 4 oC for 5 minutes. Dried microscope slides were stained with ethidium bromide (2 µg/mL in distilled H2O; 70 µl/slide), covered with a coverslip, and analyzed using a fluorescence microscope (Nikon, Japan) at a 200× magnification with epifluorescence and a rhodamine filter (at an excitation wavelength of 546 nm and a barrier of 580 nm). In each sample, 100 cells were randomly scored by eye on a scale of 0–4 according to the intensity of the fluorescence in the comet tail, where 0 indicated no tail, 1 indicated a comet tail that was less than half the width of the nucleus, 2 indicated a comet tail equal to the width of the nucleus, 3 indicated a comet tail longer than the nucleus, and 4 indicated a comet tail that was greater than twice the width of the nucleus. This type of cell scoring is proven to be as accurate and precise as computerized image analyses.

Micronucleus Assay

A micronucleus (MN) assay was performed to Fenech et al.'s specifications (25) with the minor modifications that follow: cytochalasin B (at a final concentration of 4.5 µg/ml) was added to the PBLC-SS at the 44th hour of the incubation period to block cytokinesis and to detect the number of dividing micronucleated PBLC. At hour 72, the incubation period was discontinued, and the PBLC were treated at 4–8 oC with 0.075 M of cold KCl solution as a hypotonic solution for 5 minutes and with a cold methanol–acetic acid (3: 1, v/v) as a fixative solution for 20 minutes. This fixation stage was repeated three times

after the hypotonic treatment. Then, the suspension cells were dropped onto slides, airdried at room temperature, and stained with 5% Giemsa for 15 minutes. Microscopic evaluation of the slides was carried out according to Fenech et al.'s criteria using a light microscope (Olympus BX51, Tokyo, Japan) at 1000× magnification (25). For each concentration, 1000 binucleated cells were counted by an experienced observer.

Apoptotic DNA Fragmentation Analysis

DNA fragmentation analysis, which is often used to detect apoptosis, was investigated to determine the type of cell death caused by AlCl3 and NSE. DNA fragmentation analysis of the AlCl3 and NSE-treated PBLC was conducted with a Millipore Apoptotic DNA Ladder Detection Kit (USA) after 72 hours of incubation. The protocol was implemented as described in the manufacturer's instructions.

Cell Viability Assay Using Methyl-Thiazol-Tetrazolium Test

The cytotoxicity of AlCl3 and NSE was determined according to Mosmann's specifications (26) for the methylthiazol-tetrazolium (MTT) assay with minor alterations (24). The PBLC were seeded in 24-well plates at a density of 3 × 105 cells/well. As in the experimental design, the PBLC were also exposed to AlCl3 and NSE for 72 hours. After 72 hours of exposure, the medium was removed, and the cells were washed with PBS. Then, MTT (5 mg/mL) was added to the wells and incubated at 37 oC for 4 hours. The insoluble formazan crystals that formed after the incubation were dissolved in DMSO, and cell viability was measured in terms of absorbance at 570 nm. The decrease in the number of living cells was described as the percentage of the control group, which was determined to be 100%.

Statistical Analysis

IBM SPSS 23.0 (Computer based software program, Chicago, IL, USA) was used to perform all analyses. Nonparametric independent group comparisons were made for statistical analyses. The Kruskal–Wallis test was used to conduct multiple comparisons, and the Mann–Whitney U test was used to make comparisons between groups and to determine if any statistical significance was found. Data were expressed as mean ± standard error (SE). All the statistical tests were two sided, and a *p* value of less than 0.05 was accepted to be statistically significant.

Results

Preliminary Studies Stage

Preliminary studies were performed using the comet assay method to investigate the genotoxic effects of AlCl3 and the anti-genotoxic efficiency of NSE against the toxic effects of AlCl3 on different concentrations of PBLC *in vitro* (Figure 1). Initially, the PBLC were exposed to different concentrations of Al, and 100 µM of AlCl3 significantly increased DNA damage in comparisons with the control group ($p < 0.05$). Subsequently, different concentrations of NSE were used to investigate the protective effects of NSS against 100 µM of AlCl3-induced DNA damage, and NSE was shown to have a significant genoprotective effect at 0.1, 1, and 2 µg/mL when compared to the Al-100 group ($p < 0.05$ for all). However, at high concentrations, it was observed that DNA damage to NSE (> 2 µg/mL) had greatly increased when compared to the control group ($p < 0.05$).

Main Studies Stage

The study maintained 100 µM of AlCl3 and 1 µg/mL concentrations of NSE as a result of the preliminary studies.

DNA Damage by Comet Assay

At this stage, the protective effect of 1 µg/mL of NSE against DNA damage caused by 100 µM of AlCl3 in PBLC was re-examined using the comet assay method (Figure 1). In this set, NSE was shown to have a protective effect against Al toxicity on DNA ($p < 0.05$).

Evaluation of MN

A MN assay was performed on the PBLC to investigate the preventive effect of 1 µg/mL of NSE against 100 µM of AlCl3-induced cytogenetic damage (Figure 2). In this set of the experiment, it appeared that AlCl3 significantly increased MN induction when compared to the control group ($p < 0.05$). Nevertheless, a significant reduction in the AlCl3-induced MN was observed in the PBLC that had been pre-treated with NSE ($p < 0.05$).

DNA Fragmentation Analysis of the PBLC

DNA fragmentation analysis of the PBLC with gel electrophoresis was ascertained to determine the type of cell deaths that were caused by AlCl3 and NSE. The results revealed that although the genomic DNA from the PBLC that had been exposed to 100 µM of AlCl3 or 50 µM of H2O2 were decomposed into a highly dispersed pattern, a typical DNA ladder which is a hallmark of apoptotic cell death did not appear clearly in the electrophoresed gel. However, neither the DNA cleavage nor ladder pattern appeared in those of the negative control group, and the 100 µM of AlCl3-exposed PBLC that were pre-treated with 1 µg/mL concentrations of NSE (Figure 3).

Cell Viability

To investigate the effects of AlCl3 and NSE, we performed cytotoxicity studies on the PBLC using a MTT assay as a measure of cell viability. As presented in Figure 4, AlCl3 and NSE showed a dose-dependent cytotoxic effect (> 50 µM and > 25 µg/mL, respectively) ($p < 0.001$ for all). However, at low concentrations (10–25 µM of AlCl3 and 1–10 µg/mL of NSE), neither showed any effect on cell viability. IC50 values for AlCl3 and NSE were also identified and determined to be 276 µmol/L and 67.12 µg/mL, respectively. The cell viability of the 100 µM of AlCl3-exposed PBLC that were pre-treated with 1

µg/mL of NSE are also illustrated in Figure 4. In this set, 1 µg/mL of NSE did not prevent the cytotoxicity that had been caused by 100 µM of AlCl3 (p>0.05).

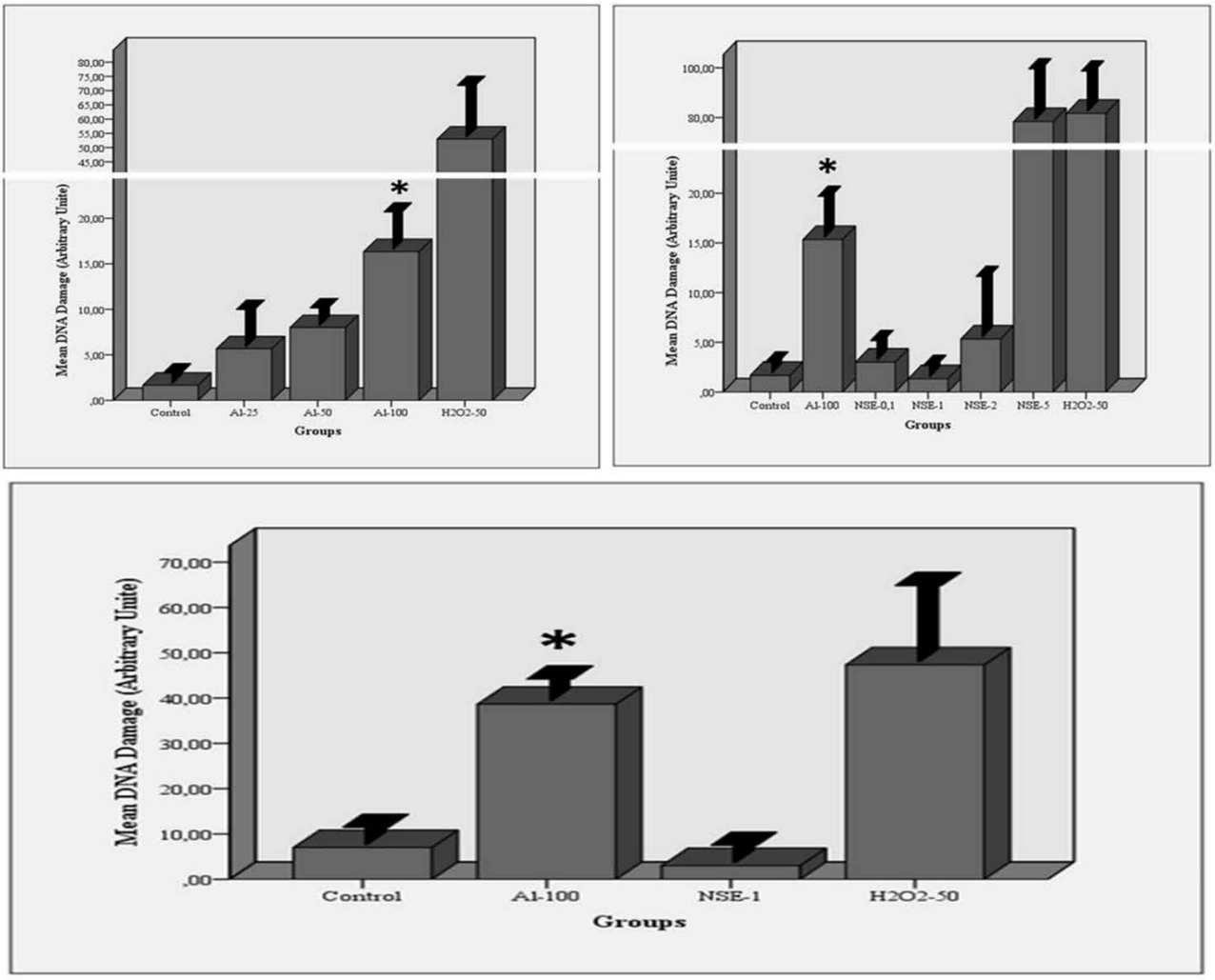

Figure 1. Mean DNA Damage values in human PBLC treated with different concentration of AlCl3 and NSE for 72 hours *in vitro*. PBLC-SS; PBLC-Suspended solution, Control (PBLC-SS), Al-25 (PBLC-SS + 25 µM of AlCl3), Al-50 (PBLC-SS + 50 µM of AlCl3), Al-100 (PBLC-SS + 100 µM of AlCl3), NSE-0.1 (PBLC-SS + 100 µM of AlCl3 + 0.1 µg/mL of NSE), NSE-1 (PBLC-SS + 100 µM of AlCl3 + 1µg/mL of NSE), NSE-2 (PBLC-SS + 100 µM of AlCl3 + 2 µg/mL of NSE), NSE-5 (PBLC-SS + 100 µM of AlCl3 + 5 µg/mL of NSE), and H2O2-50 (PBLC-SS + 50 µM of H2O2). Values are represented as mean ± standard error. * indicates a statistically significant difference between the Al-100 group and Control, NSE-0.1 and NSE-1 groups (p< 0.05).

Discussion

Living organisms are frequently and significantly exposed to Al through air, water, drugs, foodstuffs, and cosmetic products (2). Chronic exposure to Al increases free radical production, which can result in oxidative damage to DNA, lipids, and proteins (11). Fortunately, organisms are equipped with DNA repair mechanisms that protect their genetic material against harmful environmental factors, like chronic Al exposure. If the balance between DNA damage and its repair mechanisms is disturbed, DNA damage can result in neurotoxicity (1), immunotoxicity (4), hepatotoxicity (8), genetic instability, controlled cell death, and certain types of cancer (5). For this reason, several natural plants with antioxidant, antigenotoxic, and free radical scavenging properties are essential in the treatment of these disorders. Therefore, this study investigated the anti-genotoxic and anti-cytotoxic effects of NSE against AlCl3-induced DNA damage and cytotoxicity in PBLC.

The genoprotective effect of NSE was investigated by using the comet assay method, one of the standard methods for evaluating DNA damage that involves single- and double-stranded DNA breaks (24). NSE at a concentration of 1 µg/mL was found to significantly decrease the DNA-damaging effect of Al toxicity (Figure 1). As in the present study, several investigations have shown that NSS and TQ have genoprotective and free radical scavenging properties (18,22,27,28). However, in the current

study, higher concentrations of NSE also resulted in DNA damage (>2 µg/mL). Similar to this result, Zubair et al. indicated that TQ can cause oxidative DNA breakage in human PBLC through mobilization of endogenous copper ions (29).

To evaluate the anti-genotoxic activity of NSS against Al toxicity, an MN assay that examined clastogenic potential was investigated. Studies have demonstrated that Al treatments can induce damage to genetic material and thereby enable the formation of binucleated micronucleus (BNMN) (4,30). However, as far as we know, this is the first report to study the anti-genotoxic activity of NSS using a MN test. In the present study, NSS was found to significantly reduce the clastogenic effect of Al (Figure 2).

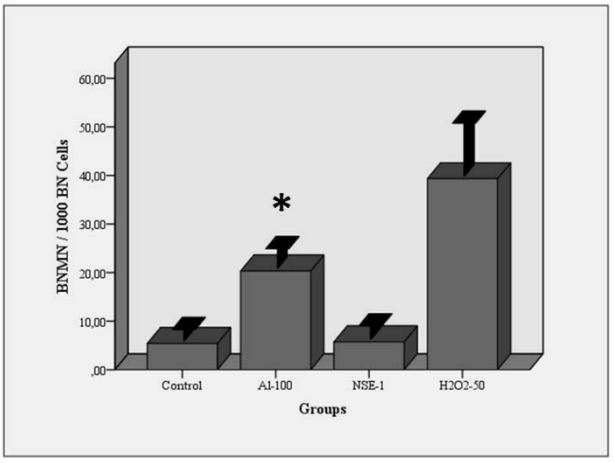

Figure 2. BNMN/BN ratio in human PBLC treated with 100 µM of AlCl3 and 1 µg/mL of NSE for 72 hours *in vitro*. BN; Binucleated cells, BNMN; Binucleated Micronucleus cells, Control (PBLC-SS), Al-100 (PBLC-SS + 100 µM of AlCl3), NSE-1 (PBLC-SS + 100 µM of AlCl3 + 1µg/mL of NSE), and H2O2-50 (PBLC-SS + 50 µM of H2O2). Values are represented as mean ± standard error. * indicates a statistically significant increase of Al-100 compared to Control and NSE-1 ($p < 0.05$).

One of the main characteristics of apoptosis are fractures to the nuclear chromatin of DNA. These fractures can lead to 180 bp to 200 bp fragments, which are presented as ladder patterns in agarose gel electrophoresis (31). However, irregularly degraded DNA in necrotic cells can be illustrated as irregular and vague patterns in agarose gel electrophoresis. Thus, apoptotic and necrotic cell deaths can be distinguished through patterns of fragmented DNA (32). In this experiment, while Al did not induce a typical DNA ladder pattern, an irregular and vague DNA pattern was clearly observed in the PBLC that had been exposed to 100 µM of AlCl3 or 50 µM of H2O2. This finding is inconsistent with the report by Miao Li et al., which demonstrated that the Al treatment of rat spleen lymphocyte resulted in DNA fragmentation in the form of apoptotic ladder patterns (32). In addition, we noticed that NSS significantly prevented DNA cleavage and DNA ladder patterns in the PBLC that had been pre-treated with 1 µg/mL of NSE (Figure 3).

A MTT assay was used to investigate whether NSE influenced Al-induced cytotoxicity. After 72 hours of treatment the IC50 values of AlCl3 and NSE on the PBLC were found to be 276 µmol/L and 67.12 µg/mL, respectively. Although concentrations of NSE as high as 5 µg/mL did not affect the viability of the PBLC, NSE started to become toxic and the cell viability began to decrease at greater concentrations. Several *in vitro* studies have also indicated that NSE and its active component, TQ, induce apoptosis in cancer cell lines (19,33,34). Similarly, Al has been shown to have apoptotic and necrotic effects in a number of cell cultures and animal studies (35,36). In the present study, NSE was found to have no preventive effect against AlCl3-induced cytotoxicity in PBLC (Figure 4).

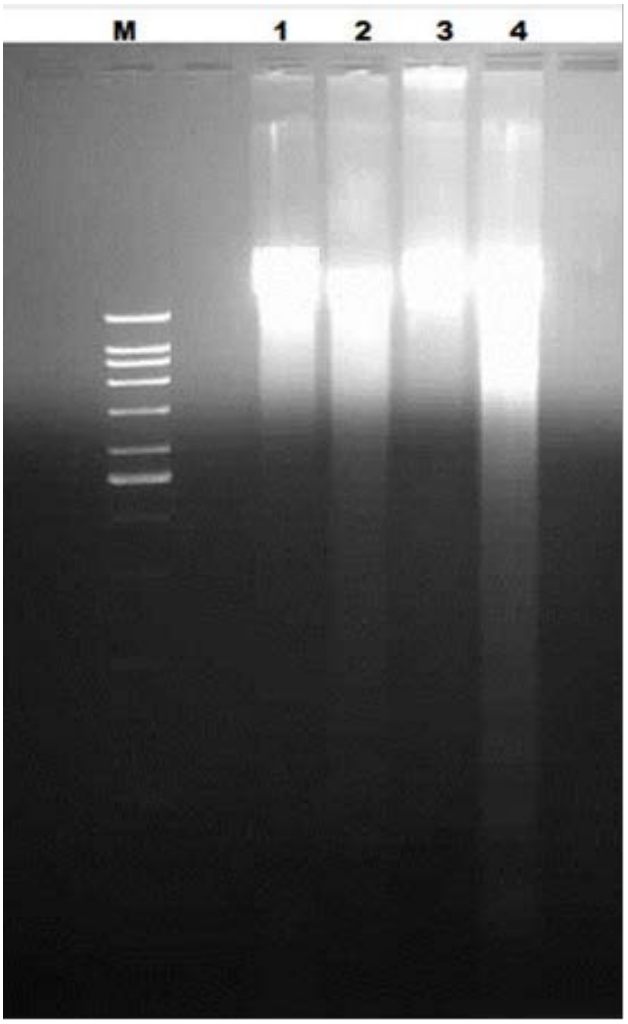

Figure 3. Apoptotic DNA fragmentation analysis of human PBLC treated with 100 µM of AlCl3 and 1 µg/mL of NSE for 72 hours *in vitro*. 1; Control (PBLC-SS), 2; Al-100 (PBLC-SS + 100 µM of AlCl3), 3; NSE-1 (PBLC-SS + 100 µM of AlCl3 + 1µg/mL of NSE), and 4; H2O2-50 (PBLC-SS + 50 µM of H2O2).

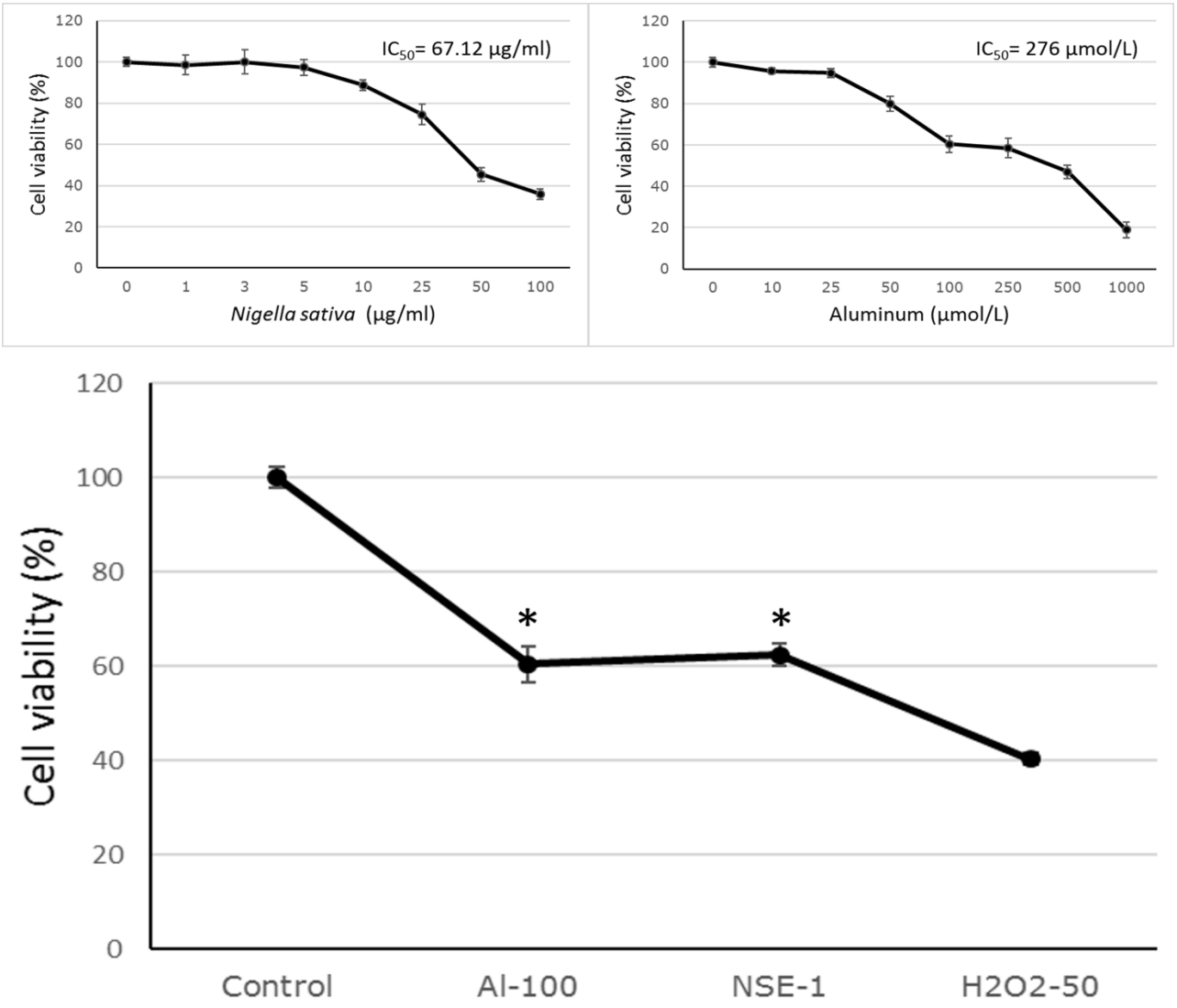

Figure 4. Cell viability ratio in human PBLC treated with different concentration of AlCl3 and NSE for 72 hours *in vitro*. Control (PBLC-SS), Al-100 (PBLC-SS + 100 µM of AlCl3), NSE-1 (PBLC-SS + 100 µM of AlCl3 + 1µg/mL of NSE), and H2O2-50 (PBLC-SS + 50 µM of H2O2). Values are represented as mean ± standard error. * indicates a statistically significant increase of Al-100 and NSE-1 compared to Control ($p < 0.05$).

Limitations

This study is limited in two ways. First, the study gathered no support from experimental animal studies. Second, a chemical composition analysis of NSE could not be performed.

Conclusions

The present investigation demonstrated that low doses of NSE (1 µg/mL) have anti-necrotic, anti-apoptotic, anti-genotoxic, and anti-clastogenic impacts against the toxic effects of Al in PBLC. However, higher concentrations (> 2 µg/mL) of NSE were found to induce DNA damage in PBLC. These findings suggest that exposure to Al in daily life should be diminished and that NSS should be consumed in low quantities.

References

1. Maya S, Prakash T, Madhu K Das, Goli D. Multifaceted effects of aluminium in neurodegenerative diseases: A review. Biomedicine and Pharmacotherapy. 2016;83:746-54.
2. Celik H, Celik N, Kocyigit A, Dikilitas M. The relationship between plasma aluminum content, lymphocyte DNA damage, and oxidative status in persons using aluminum containers and utensils daily. Clinical Biochemistry. 2012;45(18):1629-33.
3. Bhadauria M. Combined treatment of HEDTA and propolis prevents aluminum induced toxicity in rats. Food and Chemical Toxicology. 2012;50(7):2487-95.
4. Paz LNF, Moura LM, Feio DCA, Cardoso M de SG, Ximenes WLO, Montenegro RC, et al. Evaluation of in vivo and in vitro toxicological and genotoxic potential of aluminum chloride. Chemosphere. 2017;175:130-7.
5. Klotz K, Weistenhofer W, Neff F, Hartwig A, van Thriel C, Drexler H. The Health Effects of Aluminum Exposure. Deutsches Arzteblatt international. 2017;114(39):653-9.
6. Abdel Moneim AE. Evaluating the potential role of pomegranate peel in aluminum-induced oxidative stress and histopathological alterations in brain of female rats. Biological Trace Element Research. 2012;150(1-3):328-36.

7. Shati AA, Elsaid FG, Hafez EE. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup. Neuroscience. 2011;175:66-74
8. Zhu Y, Han Y, Zhao H, Li J, Hu C, Li Y, et al. Suppressive effect of accumulated aluminum trichloride on the hepatic microsomal cytochrome P450 enzyme system in rats. Food and Chemical Toxicology. 2013;51(1):210-4.
9. Kumar V, Gill KD. Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Archives of Toxicology. 2009;83:965-78.
10. Prakash A, Shur B, Kumar A. Naringin protects memory impairment and mitochondrial oxidative damage against aluminum-induced neurotoxicity in rats. International Journal of Neuroscience. 2013;123(9):636-45.
11. Bouasla I, Bouasla A, Boumendjel A, Messarah M, Abdennour C, Boulakoud MS, et al. Nigella sativa Oil Reduces Aluminium Chloride-Induced Oxidative Injury in Liver and Erythrocytes of Rats. Biological Trace Element Research. 2014;162(1-3):252-61.
12. SanJuan-Reyes N, Gómez-Oliván LM, Galar-Martínez M, García-Medina S, Islas-Flores H, González-González ED, et al. NSAID-manufacturing plant effluent induces geno- and cytotoxicity in common carp (Cyprinus carpio). Science of The Total Environment. 2015;530-531:1-10.
13. Havakhah S, Sadeghnia HR, Hajzadeh M-A-R, Roshan NM, Shafiee S, Hosseinzadeh H, et al. Effect of Nigella sativa on ischemia-reperfusion induced rat kidney damage. Iranian journal of basic medical sciences. 2014;17(12):986-92.
14. Farooqui Z, Ahmed F, Rizwan S, Shahid F, Khan AA, Khan F. Protective effect of Nigella sativa oil on cisplatin induced nephrotoxicity and oxidative damage in rat kidney. Biomedicine and Pharmacotherapy. 2017;85:7-15.
15. Canayakin D, Bayir Y, Kilic Baygutalp N, Sezen Karaoglan E, Atmaca HT, Kocak Ozgeris FB, et al. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. Pharmaceutical biology. 2016;0209:1-10.
16. Sarman H, Bayram R, Benek SB. Anticancer drugs with chemotherapeutic interactions with thymoquinone in osteosarcoma cells. European Review for Medical and Pharmacological Sciences. 2016;20(7):1263-70.
17. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific Journal of Tropical Biomedicine. 2013;3(5):337-52.
18. Usta A, Dede S. The effect of thymoquinone on nuclear factor kappa B levels and oxidative DNA damage on experimental diabetic rats. Pharmacognosy Magazine. 2017;13(51):458.
19. Taha MME, Sheikh BY, Salim LZA, Mohan S, Khan A, Kamalidehghan B, et al. Thymoquinone induces apoptosis and increase ROS in ovarian cancer cell line. Cellular and Molecular Biology. 2016;62(6):97-101.
20. Aycan IÖ, Tüfek A, Tokgöz O, Evliyaoğlu O, Firat U, Kavak GÖ, et al. Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. International Journal of Surgery. 2014;12(3):213-8.
21. Seif AA. Nigella Sativa reverses osteoporosis in ovariectomized rats. BMC Complementary and Alternative Medicine. 2014;14(1):22.
22. Mousavi SH, Tayarani-Najaran Z, Asghari M, Sadeghnia HR. Protective effect of Nigella sativa extract and thymoquinone on serum/glucose deprivation-induced PC12 cells death. Cellular and Molecular Neurobiology. 2010;30(4):591-8.
23. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental cell research. 1988;175(1):184-91.
24. Kocyigit A, Koyuncu I, Taskin A, Dikilitas M, Bahadori F, Turkkan B. Antigenotoxic and antioxidant potentials of newly derivatized compound naringenin-oxime relative to naringenin on human mononuclear cells. Drug and Chemical Toxicology. 2016;39(1):66-73.
25. Fenech M. The in vitro micronucleus technique. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2000;455(1-2):81-95.
26. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2):55-63.
27. Hashem MA, Mohamed WAM, Attia ESM. Assessment of protective potential of Nigella sativa oil against carbendazim- and/or mancozeb-induced hematotoxicity, hepatotoxicity, and genotoxicity. Environmental Science and Pollution Research. 2017;17:542-9.
28. Rastogi L, Feroz S, Pandey BN, Jagtap A, Mishra KP. Protection against radiation-induced oxidative damage by an ethanolic extract of Nigella sativa L. International journal of radiation biology. 2010;86:719-31.
29. Zubair H, Khan HY, Sohail A, Azim S, Ullah MF, Ahmad A, et al. Redox cycling of endogenous copper by thymoquinone leads to ROS-mediated DNA breakage and consequent cell death: Putative anticancer mechanism of antioxidants. Cell Death and Disease. 2013;4(6):1-8.
30. Lima PDL, Leite DS, Vasconcellos MC, Cavalcanti BC, Santos R a, Costa-Lotufo L V, et al. Genotoxic effects of aluminum chloride in cultured human lymphocytes treated in different phases of cell cycle. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2013;45(7):1154-9.
31. Zhang H, Shao D, Wu Y, Cai C, Hu C, Shou X, et al. Apoptotic responses of Carassius auratus lymphocytes to nodularin exposure in vitro. Fish and Shellfish Immunology. 2012;33(6):1229-37.
32. Li M, Song M, Ren LM, Xiu CY, Liu JY, Zhu Y zhu, et al. AlCl3 induces lymphocyte apoptosis in rats through the mitochondria-caspase dependent pathway. Environmental Toxicology. 2016;31(4):385-94.
33. Shahraki S, Khajavirad A, Shafei MN, Mahmoudi M, Tabasi NS. Effect of total hydroalcholic extract of Nigella sativa and its n-hexane and ethyl acetate fractions on ACHN and GP-293 cell lines. Journal of Traditional and Complementary Medicine. 2016;6(1):89-96.
34. Palaniswamy D, Nithyanantham M, Raghu P, Dwarampudi L. Antipsoriatic activity and cytotoxicity of ethanolic extract of Nigella sativa seeds. Pharmacognosy Magazine. 2012;8(32):268.
35. Saberzadeh J, Omrani M, Takhshid MA. Protective effects of nimodipine and lithium against aluminum-induced cell death and oxidative stress in PC12 cells. Iranian Journal of Basic Medical Sciences. 2016;19(11):1251-7.
36. Djouina M, Esquerre N, Desreumaux P, Vignal C, Body-Malapel M. Toxicological consequences of experimental exposure to aluminum in human intestinal epithelial cells. Food and Chemical Toxicology. 2016;91:108-16.