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NiO nanoparticles induce cytotoxicity mediated through ROS generation and impairing the antioxidant defense in the human lung epithelial cells (A549): Preventive effect of *Pistacia lentiscus* essential oil

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ABSTRACT

Nickel oxide nanoparticles (NiO NPs) have attracted increasing attention owing to potential capacity to penetrate to several human cell systems and exert a toxic effect. Elsewhere, the use of medicinal plants today is the form of the most widespread medicine worldwide. Utilizing aromatic plants as interesting source of phytochemicals constitute one of the largest scientific concerns. Thus this study was focused to investigate antioxidant and cytoprotective effects of essential oil of a Mediterranean plant *P. lentiscus* (PLEO) on NiO NPs induced cytotoxicity and oxidative stress in human lung epithelial cells (A549). The obtained results showed that cell viability was reduced by NiO NPs, who's also found to induce oxidative stress in dose-dependent manner indicated by induction of reactive oxygen species and reduction of antioxidant enzymes activities. Our results also demonstrated that PLEO contains high amounts in terpinen-4-ol (11.49%), germacrene D (8.64%), α-pinene (5.97%), sabinene (5.19%), caryophyllene (5.10%) and δ-Cadinene (4.86%). PLEO exhibited a potent antioxidant capacity by cell viability improving, ROS scavenging and enhancing the endogenous antioxidant system against NiO NPs in this model of cells. The present work demonstrated, for the first time, the protective activity of PLEO against cell oxidative damage induced by NiO NPs. It was suggested that this plant essential oil could be use as a cells protector.

1. Introduction

The rapid development of nanotechnology has exposed man and his environment to a number of exotic metals. In the recent years, overgrowing nanotechnology industries have led to the large-scale production of engineered nanoparticles (NPs), especially the metal NPs, for various uses [1]. Nanoparticles are defined as particles with a diameter of less than 100 nm in one structural dimension. Compared to the same materials with micrometer scale dimensions, nanomaterials have specific properties, such as small size, large surface area, shape, and special structure [2].

NPs of many different metal oxides are currently being used in numerous applications, we give as examples: magnetic resonance imaging, drug delivery, electronics, catalysis, optical devices [3,4]; as well as consumer products like cosmetics, sunscreens, sports accessories, inks and pigments, etc. [5]. Due to the growing number of applications and the recent development of combustion-based engine transportation [6], there is an increasing risk for human, environment and exposure to NPs. Their potential toxicological impacts are still a matter of investigation and our actual knowledge on the effects of nano-sized contaminants on biological systems remains incomplete [7]. Their use also (which is still largely unregulated) has become a recognized social health problem because the inhalation, dermal absorption or ingestion of particles of various sizes and compositions leads to increased rates of chronic respiratory and cardiovascular diseases [8].

Nickel oxide nanoparticales (NiO NPs) have attracted increasing attention owing to potential use in a variety of applications such as catalysts, solar cells, light-emitting diodes, lithium-ion batteries, resistive random access memory, electrochemical sensors and biosensors [9]. Therefore, increasing use of NiO NPs necessitates an improved understanding of their potential impact on the environment and human health. Besides, toxic effects of NiO NPs have been investigated in

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Table 1
Preliminary screening of leaves of *P. lentiscus*.

Chemical groups	*P. lentiscus*
Anthocyanins	+
Leuco-Anthocyanins	+
Alkaloids	−
Flavonoids	+
Tannins	+
Terpenoids	+
Steroids	−

different human cell systems, mainly on liver and airway cells [10].

Oxidative stress, which is an imbalance between ROS (reactive oxygen species) production and the antioxidant systems favoring a ROS excess, has been identified as a common mechanism for cell damage. This imbalance can be amplified by aging, smoking, diseases and some kinds of food [11]. During oxidative stress, ROS are produced mainly from the mitochondrial electron transport chain. In addition, nanoparticles have been demonstrated to generate more free radicals and reactive oxygen species than larger particles, likely due to their higher surface area [12]. The inverse correlation between cell viabilities and the ROS level proved that oxidative stress is probably a key route by which nanoparticles induce cytotoxicity [11].

In recent years, focus on plant research has increased all over the world. Many plant extracts have demonstrated potent chemo-preventive properties; most of these extracts are known to exert their effects via antioxidant mechanisms either by quenching reactive oxygen species (ROS), inhibiting lipid peroxidation or by stimulating cellular antioxidant defenses [13,14].

Pistacia lentiscus L. also known as mastic tree, belongs to the family Anacardiaceae which consisting of more than eleven species [15]. It is largely distributed in the Mediterranean region ecosystems where it grows wild in Algeria, Tunisia, Morocco, Turkey, France, Spain, Italy and Greece [16]. Despite its limited distribution in the world, this plant is known internationally for several therapeutic properties. The aerial parts have been used in the treatment of hypertension and possess stimulant and diuretic properties [17]. According to Ali-Shtayeh et al. (1998) the leaves are extensively used in traditional medicine for the treatment of eczema, diarrhea, and throat infections, and as a potent antiulcer agent [18]. Several studies have also reported that essential oil from aerial parts possesses appreciable biological properties such as antioxidant [19,20], anti-inflammatory, antimicrobial [19], anti-fungal [21], and antiatherogenic [22].

Thus, this study was designed to evaluate the antioxidant activity of PLEO using *in vitro* tests complemented with a study of their cytoprotective activity against NiO NPs induced oxidative stress in human cell culture. To our knowledge, this is the first investigation using essential oil to reduce NPs inducing cytotoxicity.

2. Material and methods

2.1. Plant materials

The leaves of *P. lentiscus* were collected at the flowering stage from the mountains of Ouled Driss in Souk ahras (36°21′N, 8°07′E, 800 m) during 2013. The sample was dried in the shade away from light at room temperature. After drying, the sample was cut to very small parts with analytical mill, used for the extraction of essential oil. The identification of the investigated specie was confirmed by Laboratory of Valorization of Natural Biological Resources (Setif, Algeria), where a voucher specimen was deposited in a botanical collection.

2.2. Phytochemical analysis

The extract of *P. lentiscus* leaves (2–3 mg/ml) was subjected to a

Table 2
Chemical composition of *Pistacia lentiscus* essential oil.

No.	Compounds	AI	Percentage (%)
1	Tricyclene	1007	0.2
2	α-Pinene	1027	**5.97**
3	α-thujene	1028	0.66
4	Camphene	1064	0.81
5	Hexanal	1077	t
6	β-Pinene	1097	1.26
7	Sabinene	1115	**5.19**
8	verbenene	1118	t
9	δ-3-Carene	1144	t
10	2-Heptanone	1157	0.11
11	α-Phellandrene	1168	1.74
12	α-Terpinene	1189	1.04
13	Limonene	1199	3.14
14	2-Hexenal	1227	0.14
15	γ-Terpinene	1249	1.77
16	β-Ocimene	1251	0.10
17	Styrene	1263	t
18	o-Cymene	1272	1.94
19	α-Terpinolene	1279	0.66
20	Isopentyl isovalerate	1296	0.19
21	(E)-2-octenal	1321	t
22	2-Nonanone	1386	0.14
23	Nonanal	1391	0.10
24	β-thujone	1429	t
25	α-Cubebene	1437	0.15
26	α-Longipinene	1451	t
27	Isopentyl hexanoate	1457	0.55
28	2,4-Heptadienal	1461	0.32
29	α-Ylangene	1469	t
30	α-Copaene	1478	1.88
31	β-Bourbonene	1503	t
32	Benzaldehyde	1519	0.10
33	2-Nonanol	1533	0.15
34	β-Cubebene	1537	0.76
35	*cis*-β-Terpineol	1562	0.10
36	Pinocarvone	1574	0.21
37	1-Terpineol	1583	0.80
38	Bornyl acetate	1600	1.14
39	Caryophyllene	1621	**5.10**
40	Aromadendrene	1631	0.67
41	Terpinen-4-ol	1604	**11.49**
42	2-Decenal	1641	t
43	*trans*-Pinocarveol	1653	0.27
44	α-Humulene	1657	1.52
45	β-Farnesene	1672	0.16
46	α-Amorphene	1681	1.82
47	Viridiflorene	1685	t
48	β-Bisabolene	1726	t
49	Verbenone	1730	0.39
50	Germacrene D	1735	**8.64**
51	α-Selinene	1740	0.55
52	β-Selinene	1743	0.51
53	α-Muurolene	1747	1.28
54	Carvone	1752	2.72
55	Cyclopentadiene	1754	0.48
56	Naphthalene	1766	2.02
57	δ-Cadinene	1768	**4.86**
58	β-Sesquiphellandrene	1775	t
59	γ-Curcumene	1779	0.41
60	Myrtenol	1794	0.39
61	2-Tridecanone	1804	0.47
62	Germacrene B	1808	t
63	cis-(Z)-α-Bisabolene epoxide	1814	0.16
64	*cis*-Calamenene	1819	0.21
65	*trans*-Carveol	1836	0.26
66	p-Cymen-8-ol	1851	0.82
67	*exo*-2-Hydroxycineole	1858	t
68	*cis*-Carveol	1867	0.15
69	α-Calacorene	1902	0.23
70	β-Ionone	1927	t
71	Caryophyllene oxide	1961	1.76
72	Salvial-4(14)-en-1-one	1985	0.17
73	β-Oploplenone	2017	0.12
74	Globulol	2061	t

(*continued on next page*)

Table 2 (*continued*)

No.	Compounds	AI	Percentage (%)
75	Spathulenol	2114	1.12
76	τ-Muurolol	2177	0.72
77	δ-Cadinol	2190	0.47
78	Nonanoic acid	2197	0.28
79	α-Bisabolol	2211	0.28
80	α-Cadinol	2221	1.27
81	Acenaphthylene	2237	0.47
82	Dehydroaromadendrene	2283	0.41
83	Alloaromadendrene oxide	2326	0.15
84	12-Norcyercene-B	2345	0.23
85	E,E-Farnesal	2366	0.30
86	Vilgarol B	2416	t
87	Benzoic acid	2448	0.64
88	Ascabiol	2609	0.16
89	Palmitic acid	2940	0.14
Total			85.59
Monoterpene hydrocarbons			24.48
Sesquiterpene hydrocarbons			29.16
Monoterpenes oxygenated			17.6
Sesquiterpenes oxygenated			6.52
Other			7.83

AI: Arithmetic Index; t: trace (< 0.1%).
Bold numbers in table are the main componds present in Pstacia lentiscus essential oil (PLEO).

preliminary phytochemical analysis for the detection of different chemical groups by using different tests and the results are presented in Table 1 [23].

2.3. Extraction, isolation and identification of PLEO

100 g of leaves were separately hydrodistilled for 3 h using a clevenger-type apparatus. Then the essential oil was dried using anhydrous sodium sulphate and stored in sterile glass vial at 4 °C until analyses. The yield of oil was calculated depending on the fresh weight of plant materials.

2.4. GC analysis

GC analysis was performed on a 6890 series instrument (Agilent Technologies, Palo Alto, CA, USA) standard model with Flame Ionization Detectors (FID) using the following conditions: fused silica capillary column with a polar stationary phase HP-INNOWax (30 m × 0.25 mm i.d, 0.25 μm film, polyethylene glycol). Helium used as a carrier gas at a flow rate of 0.5 ml/min, injector and detector temperature are 250 and 280 °C, respectively. The splitless injection mode was used with injection volume of 1 μl diluted in hexane 1%. The oven temperature was programmed at 60 °C for 8 min, and then progressed to 270 °C at 2 °C/min.

2.5. GC–MS analysis

GC/MS (EI mode, scan range of *m/z* 50–550 and ionization energy of 70 eV) data were measured on the same gas chromatograph coupled with MSD 5975. MS source temperature at 230 °C; MS quadrapole temperature at 150 °C; MSD transfer line was maintained at 280 °C. The HP-INNOWax capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was directly coupled to the MS; the other parameters were the same as with GC analysis.

Identification of components was made on the basis of their Arithmetic Index (AI) calculated following Van den Dool and Kratz [24] using a series of *n*-alkane (C_{10}-C_{30}) mixtures, and by computerized matching of the acquired mass spectra with those stored in the data bank (Wiley/NBS library) and the literature [25–27].

2.6. Antioxidant activity assays

DPPH and superoxide free radicals scavenging activity was evaluated according to Hatano et al. [28] and Beyer and Fridovich [29], respectively. The sample concentration providing 50% inhibition (IC_{50}) was calculated and reported as mean ± SD. Further, the ABTS assay was carried out utilizing the method of Re et al. [30]. TEAC value was calculated from the Trolox standard curve and expressed as Trolox equivalent (μmol/mg). The reducing power of the PLEO was determined according to reported method and expressed as Gallic acid equivalent (μg/g extract) [31]. Ascorbic acid and BHT served as positive control in all the previous assays, the experiments were also performed in triplicate and mean values recorded.

2.7. Nickel nanoparticles and reagents

Nickel oxide (NiO) nanopowder (Product No. 637130, particle size: < 50 nm and purity: 98,8% trace metals basis), MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide]), DCFH-DA (2,7-dichlorofluorescin diacetate), Dulbecco's modified eagle's medium (DMEM), hank's balanced salt solution (HBSS), fetal bovine serum (FBS), penicillin–streptomycin, trypsin, phosphate buffered saline (PBS) and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). SOD and Catalase enzyme assay kits were obtained from Bio-Vision Inc. (Milpitas, California, USA). All other chemicals used were of the highest purity available from commercial sources.

2.8. Nickel oxide nanoparticles characterization

Crystalline nature of NiO NPs was characterized by X-ray diffraction (XRD) using Shidmadzu XRD-6000 Diffractometer at room temperature, while shape and size were determined by field emission transmission electron microscopy (TEM, JEM- 2100F, JEOL Inc., Japan) at an accelerating voltage of 120 kV. On the other hand, dynamic light scattering (DLS) (Nano-ZetaSizer-ZS, Malvern Instruments, UK) was used to determine the hydrodynamic size and Zeta potential of the NiO NPs as described by Ahamed et al. (2013) [32].

Table 3
Antioxidant effect on DPPH radicals, superoxide radicals, scavenging ability on ABTS radicals and reducing power of *P. lentiscus* essential oil.

	Scavenging ability on DPPH radicals (IC_{50} μg/mL)	Scavenging ability on superoxide radicals (IC_{50} μg/mL)	Scavenging ability on ABTS radicals (μmol Trolox/mg)	Reducing power ability (μg GAE equivalent/g)
PLEO	640 ± 0.16	760 ± 0.125	0.247 ± 0.023	153.33 ± 32.44
Ascorbic acid	40	120 ± 0.005	1.172 ± 0.016	731.33 ± 24.44
BHT	58 ± 0.003	108 ± 0.002	1.072 ± 0.014	620 ± 29.61

Values are expressed as mean ± SD of three measurements. GAE: acid galic.

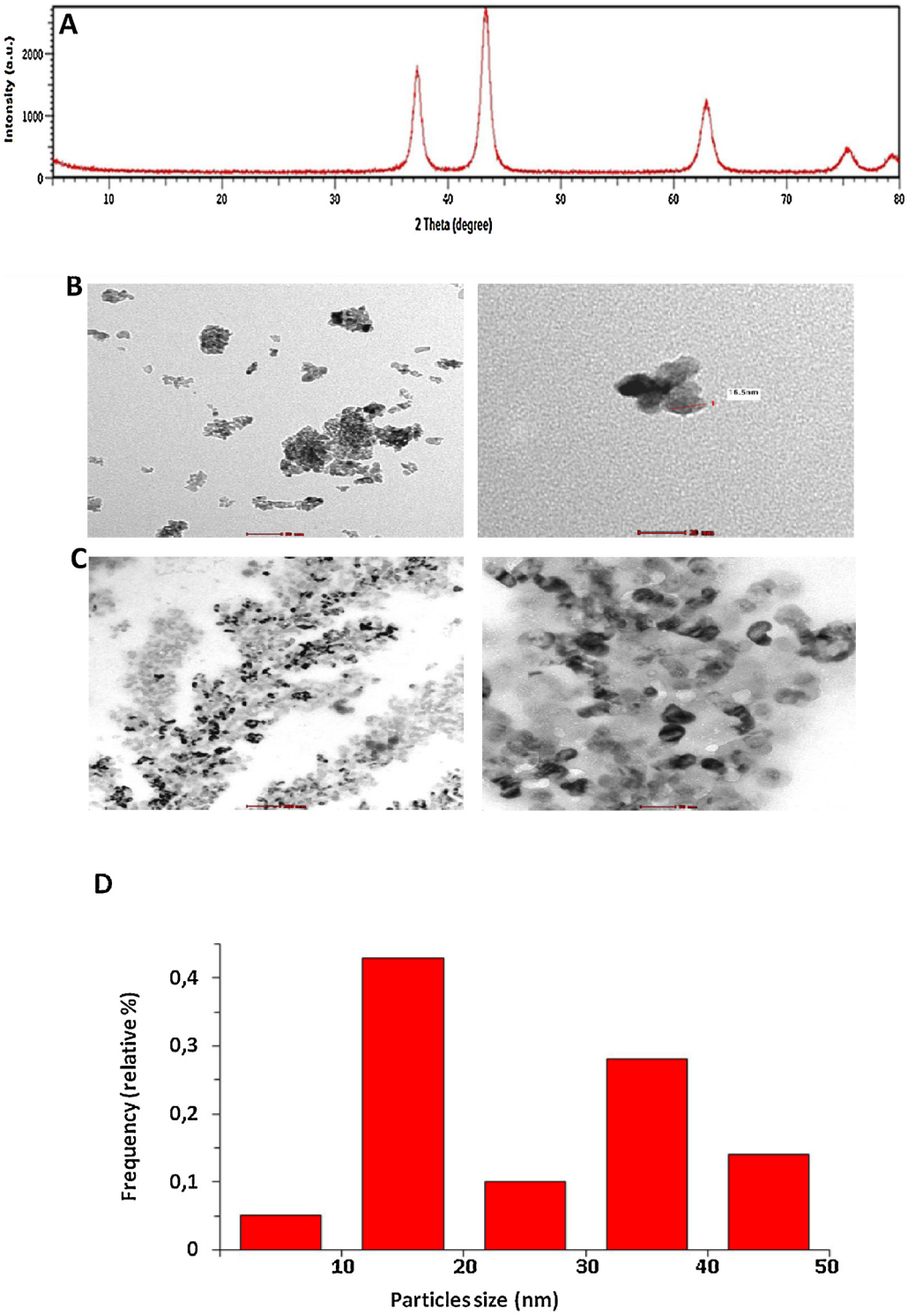

Fig. 1. Characterization of NiO NPs. (A) X-ray diffraction pattern, (B and C) transmission electron microscopy image in deionized water (50, 20 nm) and in full DMEM (200, 50 nm), respectively and (D) frequency of size distribution.

2.9. Cell culture and treatments

The human lung epithelial cells (A549) were cultured in DMEM medium supplemented with 10% FBS and 100 U/ml penicillin–streptomycin at 5% CO_2 and 37 °C. At 85% confluence, cells were harvested utilizing 0.25% trypsin and were sub-cultured into 75 cm^2 flasks, 96-well or 12-well plates according to selection of experiments. Cells were allowed to attach the surface for 24 h prior to treatment. NiO NPs were suspended in cell culture medium and diluted to appropriate concentrations (0.5, 10, 20, 40, 60, 80, 100 µg/ml) for 24 h. The appropriate dilutions of NiO NPs were then sonicated using a sonicator bath at room temperature for 10 min at 40W to avoid nanoparticles agglomeration prior to administration to the cells. Cells were treated with PLEO at concentration of 60 and 80 µg/ml for 24 h before exposure to NiO NPs (80 and 100 µg/ml). Essential oil samples were dissolved in DMSO and diluted in the medium (final concentration of DMSO in the medium was < 0.3%)

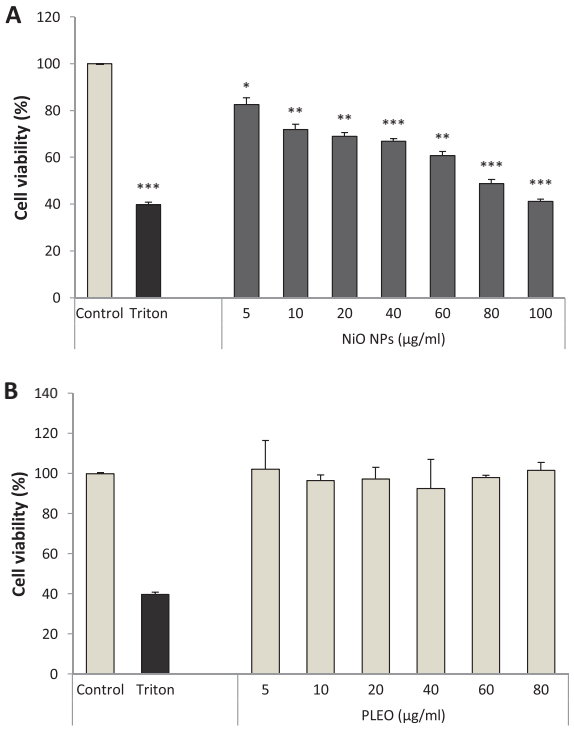

Fig. 2. (A) NiO NPs induced cytotoxicity in A549 cells. Cells were exposed to different concentrations of NiO NPs for 24 h. (B) Effects of PLEO on cell viability. A549 cells were treated with different concentrations of essential oil samples for 24 h also. Triton was used as a negative control. Data represented are mean ± SD of three independent experiments. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ treatments compared to control.

2.10. Cell viability and measurement of ROS

Viability of cells after exposure to NiO NPs was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] assay as described by Mosmann (1983) [33], whereas the production of intracellular ROS was measured using 2,7-dichlorofluorescin diacetate (DCFH-DA) [34]. Cells were seeded in 96-well plates at a concentration of 1×10^4 cells/well and exposed to NiO NPs or/with PLEO at the concentrations for 24 h.

2.11. Antioxidant enzymes activities

Superoxide dismutase (SOD) and Catalase (CAT) activities were measured by commercially available kits. The cells were seeded into 12-well plates at a concentration of 7×10^5 cells/well and all measurements were performed according to supplier's recommendations.

2.12. Statistical analysis

Data are presented as mean values ± SD and the statistical analyses of the data were performed by one-way analysis of variance (ANOVA) followed by Student's *t*-test. A probability value of $p < 0.05$ was considered to denote a statistically significant difference.

3. Results and discussion

3.1. Chemical composition of the essential oil

Results of the phytochemical analysis indicated that leaf comprising anthocyanins, leucoanthocyanins, flavonoids, tanins and terpenoids which are shown in Table 1.

Table 2 indicates the various chemical constituents identified in

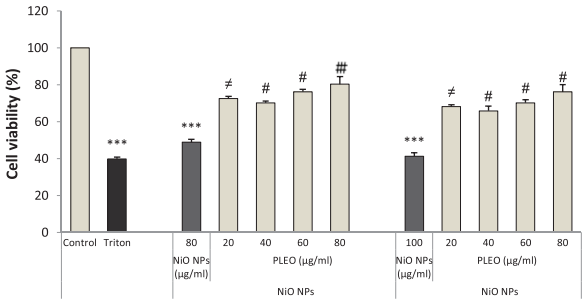

Fig. 3. Protective effect of PLEO on NiO NPs induced cytotoxicity. A549 cells were treated with different concentrations of essential oil for 24 h followed by NiO NPs for 24 h. Triton was used as a negative control. Data represented are mean ± SD of three independent experiments. $***p < 0.001$ treatments compared to control; $\neq p < 0.05$; $\#p < 0.01$; $\#\#p < 0.001$ compared to NiO NPs.

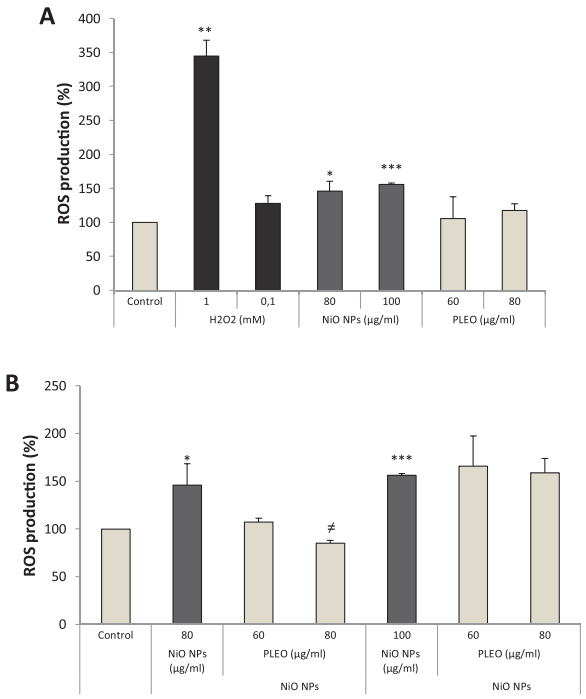

Fig. 4. (A) Effects of NiO NPs and PLEO on ROS production in A549 cells. Cells were treated with different concentrations for 24 h. H_2O_2 was used as a negative control. (B) PLEO attenuates NiO NPs induced ROS generation. A549 cells were treated with different concentrations of essential oils for 24 h followed by NiO NPs for 24 h also. Data represented are mean ± SD of three independent experiments. $*p < 0.05$; $***p < 0.001$ treatments compared to control; $\neq p < 0.05$ compared to NiO NPs.

PLEO. The yield of extraction was 0.42 ± 0.02% and it characterized by variety of components in less percentage. The main compounds present are terpinen-4-ol (11.49%), germacrene D (8.64%), α-Pinene (5.97%), sabinene (5.19%), caryophyllene (5.10%) and δ-Cadinene (4.86%).

The plant extractions provide an essential oil of light-brown color with a very strong and persistent aromatic-spicy odor. The PLEO yield obtained is similar to that in the literature [20,35]. Therefore, these results revealed that the two major components identified (terpinen-4-ol and germacrene D), founded with this amount for the first time, are in agreement with previous studies and confirmed the identity of the chemotypes used in this study [26,36].

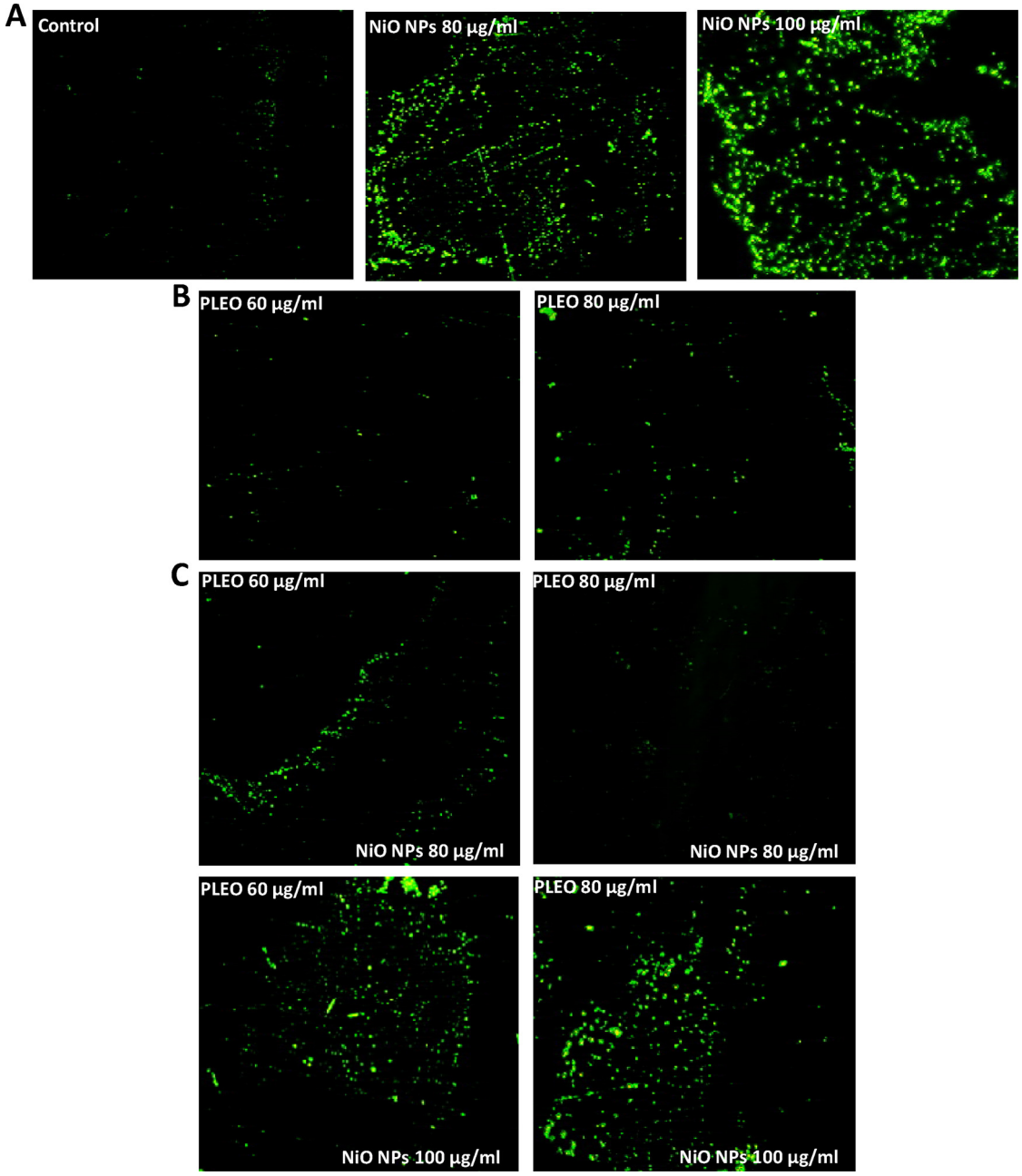

Fig. 5. Intracellular ROS generation in A549 cells. Images were captured with a fluorescence microscope (OLYMPUS IX73). Effects of NiO NPs (A) and PLEO (B) on ROS production. Cells were treated with different concentrations for 24 h. (C) PLEO attenuate NiO NPs induced ROS generation in dose-dependent manner. A549 cells were treated with different concentrations of essential oil for 24 h followed by NiO NPs for 24 h also.

3.2. Antioxidant activities

Antioxidant activities of PLEO were evaluated with four systems to establish authenticity, using tow synthetic antioxidants positive controls (BHT and Ascorbic acid). Potency was assessed by measuring the IC_{50} or Trolox/GAE equivalent and the results are reported in Table 3. The order of antioxidant capacity was Ascorbic acid > BHT > PLEO.

According to the results which have been obtained, the tested samples were able to reduce the DPPH radical and scavenging the Superoxide radical with IC_{50} equal to 640 ± 0.16 µg/ml and 760 ± 0.125 µg/ml for DPPH and SOD assay respectively. Different parts and constituents from *P. lentiscus* have been shown *in vitro* radical scavenging properties [37,38]. Using the DPPH scavenging activity assay, it was found that all of the *P. lentiscus* extracts in solvents, except for the chloroform extract, have a high radical scavenging activity (90%) equivalent to that of the standard, BHA (89%). The ethanolic and aqueous fractions from the ethyl acetate extract have high scavenging activities with values of 78 ± 0.93% and 90.29 ± 0.29%, respectively [39]. In 2007, Barra et al. [40] conducted a study which showed that the PLEO exhibited radical scavenging activity ranged between 0.52 and 4.61 mmol/l.

The highest reducing power ability (µg GAE equivalent/g) and scavenging ability on ABTS radicals (µmol Trolox equivalent/mg) were observed also in the following order; ascorbic acid > BHT > PLEO. From our data, it appears that the values in the present study were comparable to those of Ascorbic acid and BHT. While comparing our present results of this assay with the literature already published, we found very few reports on the antioxidant activity of PLEO. We are the first evaluated the reducing power, ABTS and Superoxide radicals scavenging ability of PLEO.

3.3. NiO NPs characterization

Fig. 1A shows a sharp diffraction peaks that clearly indicates the crystalline nature of NiO NPs. No peaks of impurity were found in the XRD pattern, indicating that the nanoparticles of NiO used in this study consists of ultrapure phase. The existence of strong and sharp diffraction peaks at 2θ = 37.41, 43.35, 62.95, 75.40 and 79.45 which perfectly corresponding to (111), (200), (220), (311) and (222) crystal planes, respectively (JCPDS Card No. 73-1523). Besides, the crystallite size has been estimated from XRD using the Scherer's equation [41]. The average nanocrystallite size of NiO NPs corresponding to the highest peak observed in XRD was found to be 24 nm. The typical TEM image of NiO NPs is indicated in Fig. 1B and C. TEM analyze show that the average diameter, from measuring over 70 particles, of NiO NPs was around 25 nm (further supporting the XRD results) and they have the tendency to aggregate in the solution used. Fig. 1D represents frequency of size distribution of NiO NPs. The measurements, performed by DLS, determined the average hydrodynamic size (293.86 ± 9.51 and 288.1 ± 7.04 nm) and the zeta potential (−14.06 ± 0.60 and −18.2 ± 1.10 mV) of NiO NPs in water and culture media, respectively.

3.4. MTT assay

MTT assay was undertaken in order to evaluate the effectiveness of PLEO extracted on cell viability in A549 cells stressed by NiO NPs. First we evaluated the effects of NiO NPs on A549 cells viability. Incubation with 5, 10, 20, 40, 60, 80 and 100 µg/ml for 24 h resulted in a concentration dependent decrease in cell viability, the LC_{50} was 79.83 ± 0.856 µg/ml (Fig. 2A). Based on these results NiO NPs at submaximal concentrations after 24 h, 80 and 100 µg/ml were selected in this study. Next, we found all concentrations of PLEO used in MTT assay were determined to be non-toxic for A549 cells (Fig. 2B).

Therefore, the pre-exposition for 24 h to PLEO (60 and 80 µg/ml) were the best concentrations exhibited protective effect in cell viability reduced by NiO NPs (Fig. 3).

The main findings of this assay are the LC_{50} of NiO NPs was 79.83 µg/ml and submaximal concentrations of 80 and 100 µg/ml were selected in this study. Similar results were obtained in previous findings demonstrated a dose dependent reduction of MTT-value in A549 cells treated with NiO NPs [10] or with Ni NPs [42].

According to the Natiaonal Cancer Institute (USA), vegetables crude extracts are cytotoxic considered when their IC_{50} values are less than 30 µg/ml [43]. After a large screening, PLEO (60 and 80 µg/ml) concentrations were selected due to their best actions. Whereas, the concentration of 80 µg/ml has the highest cytoprotective effect on NiO NPs reduced cell viability. In addition on the obtained results, it was not detected toxic effect in this model of human pulmonary cell line exposed to PLEO. The present study agree with the results of Remila et al. (2015) who have demonstrated that pre-trearment of THP-1 cells with *P. lentiscus* extracts for 24 h strongly inhibited H_2O_2 damage, with maximum protection at 100 µg/ml [15].

3.5. ROS production

In order to investigate the protective effect of PLEO on NiO NPs induced cytotoxicity mediated through ROS generation, A549 cells were treated with the two selected concentrations of the essential oil 24 h previous to NiO NPs. We detected a significant decrease of ROS level in cells treated with the essential oil (Figs. 4 and 5).

Oxidative stress, which is an imbalance between ROS production and the antioxidant systems favouring a ROS excess, has been identified as a common mechanism for cell damage. During oxidative stress, ROS are produced mainly from the mitochondrial electron transport chain. To minimize the damage induced by ROS, free radicals can be transformed to other less toxic molecules, for example, the superoxide anion is enzymatically converted into hydrogen peroxide by superoxide dismutase (SOD) and hydrogen peroxide may be enzymatically converted into water by catalase or glutathione peroxidase enzymes [44].

Nanoparticles have been demonstrated to generate more free radicals and ROS than larger particles, likely due to their higher surface area [12]. NiO NPs have been reported to reduce cell viability and to induce oxidative stress by depletion of glutathione and induction of reactive oxygen species in HEp-2 and MCF-7 cells [45], cell death via apoptotic pathway and ROS generation in HepG2 cells in dose-dependent manner [32], NiO NPs also increased intracellular ROS, apoptosis and necrosis in BEAS-2B and A549 cells [10], Our results confirmed that NiO NPs is toxic to A549 cells.

Moreover, we noted that pre-incubation of cells with PLEO led to decrease of ROS generation which is due to the presence of terpinen-4-ol and, probably, to synergistic effect of its components. It has been reported that plant phenols can behave as ROS scavengers, metal chelators and enzyme modulators and prevent lipid peroxidation [46]. In agreement with our data obtained, pre-treatment of THP-1 cells with *P. lentiscus* extracts for 24 h strongly inhibited H_2O_2 damage [15].

3.6. Antioxidant enzymes activities

Pre-incubation of cells with both concentrations 60 and 80 µg/ml of PLEO led to enhance the antioxidant enzymes, SOD and CAT, activities affected by NiO NPs us shown in Figs. 6 and 7.

Similarly, the NPs also induce a significant depletion of antioxidants. The accumulation of ROS, e.g. superoxide radicals (O_2·) and hydroxyl free radicals (·OH) decrease the defensive effects of cellular antioxidant enzymes, e.g. SOD, CAT [47]. Exposure of HT22 hippocampal cells to CuO NPs resulted decrease in the activity of SOD and the other detoxification enzymes which has been founded in this work [48]. In contrast, these findings suggest that pre-treatment of cells with essential oil averted cell damage by preventing the decreased activity of antioxidant enzymes, SOD and CAT, induced by NiO NPs. In keeping with our results, many previously works has described the ability of

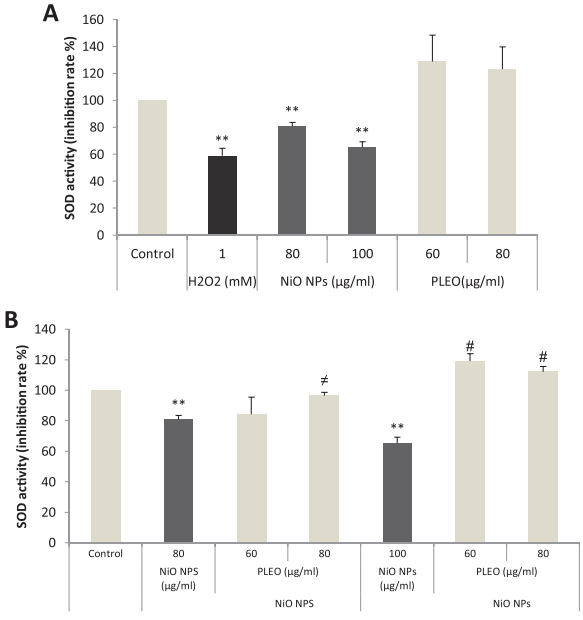

Fig. 6. (A) Effects of NiO NPs and PLEO on SOD activity in A549 cells. Cells were treated with different concentrations for 24 h. H_2O_2 was used as a negative control. (B) *P. lentiscus* essential oil attenuates NiO NPs decreased SOD activity. A549 cells were treated with different concentrations of essential oils for 24 h followed by NiO NPs for 24 h also. Data represented are mean ± SD of three independent experiments. $^{**}p < 0.01$ treatments compared to control; $\neq p < 0.05$ and $\#p < 0.01$compared to NiO.

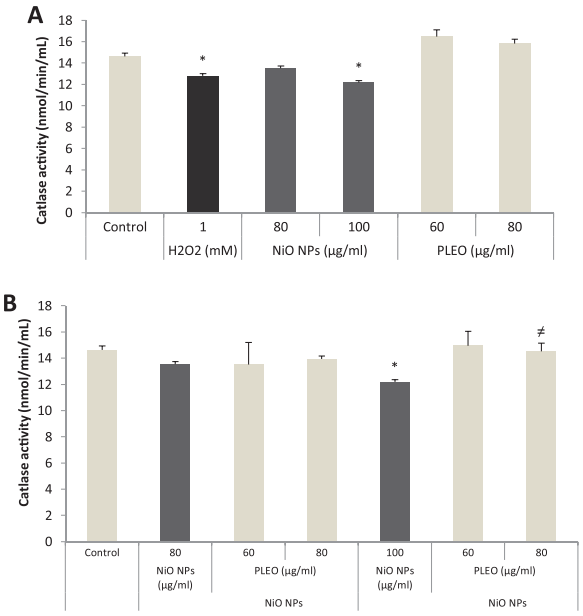

Fig. 7. (A) Effects of NiO NPs and PLEO on CAT activity in A549 cells. Cells were treated with different concentrations for 24 h. H_2O_2 was used as a negative control. (B) PLEO attenuates NiO NPs decreased CAT activity. A549 cells were treated with different concentrations of essential oils for 24 h followed by NiO NPs for 24 h also. Data represented are mean ± SD of three independent experiments. $^*p < 0.05$ treatments compared to control; $\neq p < 0.05$ compared to NiO NPs.

essential oil to modulate the endogenous enzymatic system [47,49].

4. Conclusion

The data demonstrated that NiO NPs induced cytotoxicity in a concentration-dependent manner, which the mechanism may be through ROS generation and oxidative stress. The crucial results highlight, for the first time, the antioxidant and the protective activities of essential oil of *P. lentiscus* against cell oxidative damage induced by NiO NPs. These findings also showed the guidelines in the placement of NPs in safety to promote non-hazardous applications of nanotechnology.

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