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RESEARCH ARTICLE

Effect of water-soluble PM_{10} on the production of TNF-α by human monocytes and induction of apoptosis in A549 human lung epithelial cells

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Abstract

Long-term exposure to airborne particles of 10 μm and less in size (PM_{10}) in dust can lead to increased risk of diseases such as respiratory, cardiovascular, lung cancer and atherosclerosis. The aim of the study was to evaluate the effects of water-soluble PM_{10} particles in the Middle East Dust (MED) storm in Ahvaz, Iran, on the production of TNF-α by human monocytes. In addition, we assessed the level of induction of apoptosis in isolated A549 human pulmonary epithelial cells. For this purpose, isolated human blood monocytes (250,000 to 300,000 cell/ ml) as well as isolated human pulmonary A549 epithelial cells (100,0000 cell/ ml) were exposed to various concentrations (62.5, 125, 250, 500 μg/ml) of water-soluble PM_{10} particles for different incubation periods (12, 24, 48 h). The results showed that exposure to PM_{10} particles increased the production of TNF-α in human monocytes and promoted apoptosis induction in A549 cells, in both concentration and incubation of period-dependent manner. In conclusion, airborne dust particles in Ahvaz city contain active compounds capable of increasing production of the pro-inflammatory cytokine, TNF-α, and inducing apoptosis of lung epithelial cells.

Keywords Dust · Monocyte · Apoptosis · TNF-α · A549 cell line

Introduction

Airborne particles include a mixture of solid or liquid suspended matter in the atmosphere. These particles are abundant and vary in size and chemical content, solubility, and source [27, 16]. Respirable particles are often related to particles with an aerodynamic diameter of less then or equal to 10 μm, which are called PM10 and are classified into two main groups: particles with 2.5–10 μm size and particles who's size between 1 and 2.5 μm [3]. The particle source is either natural or anthropogenic, consequently, particles contain various components including biomaterials [18, 28], chemicals such as metals [14], salt [31], mineral or organic matter, and endotoxins that can be absorb into tissues and effect the cell nucleus [2]. Prolonged inspiration of airborne dust particles leads accumulation in the lower respiratory tract resulting in increased risk of diseases such as respiratory, cardiovascular, lung cancer and atherosclerosis [5, 12]. On the other hand, short term exposure to particles suspended in the atmosphere can lead to exacerbation of respiratory diseases such as bronchitis and asthma and induce changes in the heart rate [11, 32]. Epidemiological studies suggest that, despite a wide range of geographical locations, airborne particles can increase the risk of mortality [21]. A previous in vitro study on airborne particles from different zones of Mexico city reported different pro-inflammatory and toxic effects. Particles collected from the central and northern zones of this city were more toxic and produced greater inflammatory responses (increased production of TNFa and IL-6) in cultured mouse monocytes. This differential

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toxicity was attributed to the composition and to the size of the particles [2]. Previous investigation in Ahvaz focused on the chemical composition of PM_{10} particles collected during dust storms containing soluble and insoluble particles. The soluble components were found to be more toxic on human lung epithelial cells inducing greater increase in lactate dehydrogenase activity then insoluble ones [23]. No previous study has been conducted specifically aiming to assess the pro-inflammatory and the apoptotic capacity of the soluble components of dust storm on human monocytes, TNF-α as a marker for pro-inflammation, and induction of apoptosis in A549 human lung epithelial cells.

Material and methods

Particle sampling

Particles were collected by a high volume sampler (Tisch Co.) equipped with quartz microfilters (8 × 10 in, Watman, USA), which was placed on the roof of the Health Faculty of Ahvaz University of Medical Sciences, at a height of 10 m. A stock solution of 5000 μg/ml of water soluble inorganic ions was prepared from the collected samples as described by Naimabadi et al., and stored at -70 °C until needed [23].

Cell culture

Human pulmonary epithelial cells (A549) was purchased from Pasteur Institute at National Cell Bank of Iran (NCBI). These cells were suspended in RPMI 1640 supplemented with 10% FBS, 1% penicillin, streptomycin, 2 mM L-glutamine, and incubation at 37 °C in a humidified atmosphere with 5% CO_2. Than, for each incubation, 1×10^6 cells/well were seeded into the 24-well cell culture plate and incubated for 24 h to insure that the cells adhered to the plate floor and intracellular interaction was established [33].

Blood samples were collected for the preparation of human monocytes. Blood was collected in sterile collection tubes containing heparin as an anticoagulant and was diluted 1:2 with phosphate buffer. Than, plasma and platelets were removed after Ficoll-Hypaque centrifugation (Centrifuge 30 min at 300 g), and isolated mononuclear cells were transferred to a new centrifuge tube. The isolated cells were washed 3 times with Hanks buffer, centrifuged for 10 min at 1400 rpm (400 g) at 18–20 °C [15]. In this step, the upper layer was discarded and the remaining adherent cells were resuspended in DMEM to reach the concentration of 2×10^6 cells/ ml, and 10 ml of which was transferred and incubated in a flask at 37 °C for 1 h, with 5% CO_2. In the next step, the supernatant was discharged and the adherent cells were washed twice with DMEM to remove unbound cells. Than, 10 ml fresh DMEM was added to the remaining cells and suspended the adherent cells in DMEM by gentle scraping (with a plastic scraper) and counted them. At least 95% of the isolated monocytes were viable after the vital staining with Wright- Giemsa [40].

Evaluation of the apoptotic capacity of PM_{10} water soluble dust particles on A549 cells

In order to assess the apoptotic capacity of water-soluble PM_{10} dust particles, four different concentrations (6.25, 12.5, 250 and 500 μg/ml, taken from a stock solution of 5000 μg/ml) were prepared in RPMI 1640 [1, 9]). Triplicates of the seeded Human pulmonary epithelial cells (A549) in 24 well plate(1,000,000 cells per well), were exposed to the soluble PM_{10} fractions containing 62.5, 125, 250 and 500μ g/ml for varying periods of 12, 24, and 48 h. The cells were coated with two fluorochromes (Annexin V and propidium iodide) using BD kit (Abcam, Cat number: ab14085) and were evaluated by BD flow cytometry. Finally, the data were analyzed by WIND MI software.

The production level of TNF-α in monocytes exposed to water soluble PM_{10} fraction

In order to evaluate the level of production of TNF-α in human monocytes, the isolated monocytes, in triplicates and at a density of 3×10^5 cells/well in a 96-well cell culture plate, were incubated at 37 °C for 24 hours with 5% CO_2 [17]. After this incubation time, the monocytes were exposed to different concentrations (62 0.5, 125, 250, 500 μg/ml) of water soluble PM_{10} fraction suspended in RPMI 1640 for different periods of 12, 24, 48 h. Lipopolysaccharide(LPS) from Escherichia coli (K 235, Sigma) was added in a concentration of 5 μg/ml in each incubation times as positive control [20]. After this incubation time, the supernatants were collected and stored at -70 °C and than the rate of TNF-α production was determined in the supernatant according to the protocol of the ELISA kit (eBioscience, San Diego, CA, USA). This step was repeated triplicate.

Statistical analysis

For multiple comparisons within a data set, one-way ANOVA was performed. For single comparisons, a paired sample t-test was used. P-value < 0.05 was considered significant.

Remote sensing of dust

A better understanding of the MED storm movement was provided using Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) [34]. Trajectories were calculated beginning at three different starting heights (300 m, 1000 m, and 3000 m) using the NOAA meteorological database. Due to abundant plain covering areas in the Middle East particularly

Khuzestan province we selected 300 m as the first starting height.

Aerosols include tiny liquid or solid dust and they're characteristics which can be measured by MODIS. Aerosols mount into the atmosphere from anthropogenic and natural sources. MODIS helps us to realize air mass movement and to confirm either a backward or a forward trajectory.

Results

Apoptotic impact of water soluble PM_{10} on the human cell line

A549 cell line exposure to different concentrations of water soluble PM_{10} in varying incubation times showed that the highest apoptosis was observed with the highest concentration of the water soluble fraction of the dust particles (500 μg/ml) after passing 48 h of incubation. The apoptosis rate in the studied cells was reported 21%, which was significantly higher then unexposed control cells, with 12% ($P < 0.05$). While the lowest rate of apoptosis (15%) was observed with the lowest concentration (62.5 μg/ml) after the same incubation period (Fig. 1). Generally, at each incubation, the highest apoptosis is observed at the highest concentration of dust particles, and the lowest apoptosis is at the lowest concentration of dust particles (Fig. 2).

Production of TNF-α cytokine in human monocytes exposed to water soluble PM_{10}

Following exposure of isolated human monocytes to different concentrations of water soluble PM_{10} dust particles in varying incubation times, the level of TNF-α cytokine in the supernatant of the monocytes culture medium was evaluated by sandwich ELISA. Analysis of data showed that they're was a significant difference in the production of TNF-α protein compare to none exposed control group (Fig. 3). The highest production of this cytokine in the human monocytes was 234 pg/ml which was observed at the highest concentration of dust particles (500 μg/ml) after 12 h of incubation time. However, following longer exposure (both 24 and 48 h) to this concentration, the production of this cytokine was reduced in a time-dependent manner. A similar trend was observed for other concentrations (Fig. 3). Despite this reduction trend, the overall rate of TNF-α production level, increased in parallel with an increase in the concentration of dust particles compared to none exposed control at each time point of incubation (Fig. 3).

Trajectory and MODIS analysis

Figure 4 presents the Hysplit backward trajectory of a dust storm and satellite imagery beginning at 10:00 AM (UTC) January 08th, 2013. The red line is the closest trajectory to the ground (300 m). It shows that air mass was moved from the region including Iraq and Saudi Arabia before encountering Iran. The prevailing winds in this area of the MED are westerly and Shamal winds which are the dominant cause of air mass movement. Geographically, it can be concluded that Iraq, Kuwait, and Saudi Arabia and even dried land in Khuzestan province are believed to be the short-range emission sources of studied dust [29]. Considering long-range transport from Sahara, Syria, and Jordan should not be neglected as well.

Discussion

In this study, we investigated the effects of different concentrations of water-soluble PM_{10} fractions of respirable airborne particles of the MED storm on the production of TNF-α in human monocytes cells and apoptosis induction in A549 human lung epithelial cells. The results showed that these respirable airborne particles have the capacity to stimulate and activate human monocyte cells to produce the pro-inflammatory cytokine, TNF-α. In addition, it induced apoptosis in pulmonary epithelial cells in a concentration and duration of exposure-dependent manner.

In the first step, we investigated the stimulatory capacity of respirable dust particles on human monocytes. The results showed that after exposure of human monocytes to different concentrations of water soluble dust particles TNF-α protein production level increased in a dose-dependent manner. These results come consistent with previous studies from other countries [6, 37, 39]. In our study, analysis of TNF-α protein production indicates that the highest production of TNF-α is related to the exposure of monocytes with the maximum concentration of particles (500 μg/ml) in the minimum incubation time (12 h). The highest rate of production of this cytokine at the lowest incubation time reminds us that dust particles might be able to stimulate the immune system and produce pro-inflammatory cytokines.

The results showed that in all the same concentration of dust particles, increasing incubation time had a decreasing trend in the production of TNF-α protein, it can be concluded that the production of TNF-α protein is also time dependent manner. The reduction of this cytokine by increasing the incubation time has been confirmed by another study [4, 30]. This decrease in the production of TNF-α protein indicates that TNF-α is one of the first acute phase cytokines, and increased secretion and prolongation of the release of this cytokine can trigger inappropriate inflammatory responses in the body. So, obviously the regulatory mechanisms for immune responses try to inhibit excessive TNF-α production. Studies have shown that the expression of the TNF-α gene

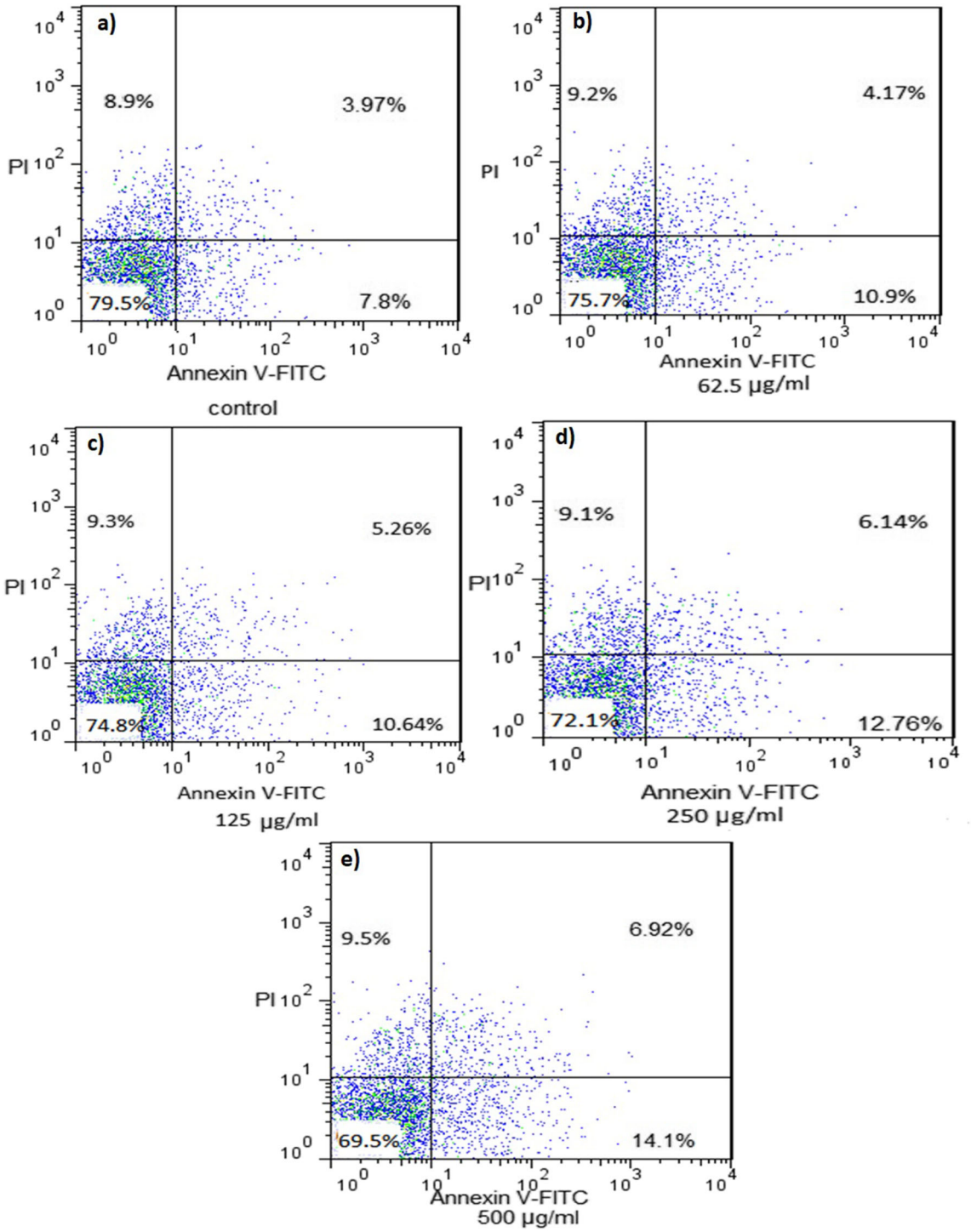

Fig. 1 A representative dot plot of flow cytometry showing the level of apoptosis of human pulmonary epithelial cells following exposure to different concentrations after 48 h of incubation

is strongly controlled at the level of transcription and stability of mRNA and prevents over-stimulation of the immune system [8, 19]. Also, previous studies have shown that the addition of TNF-α to human monocytes cell culture has led to a significant increase in the expression of the IL-10 gene compared to those not added. Induction of the gene expression of IL-10 by TNF-α is dos-dependent and increasing TNF-α level also increases the expression and production of IL-10. On the other hand, IL-10¸ which is produced by the affect of TNF-α on the monocytes suppresses the expression and production of TNF-α, which is considered as self-regulatory or negative feedback [41]. Other studies have shown that IL-4 is another

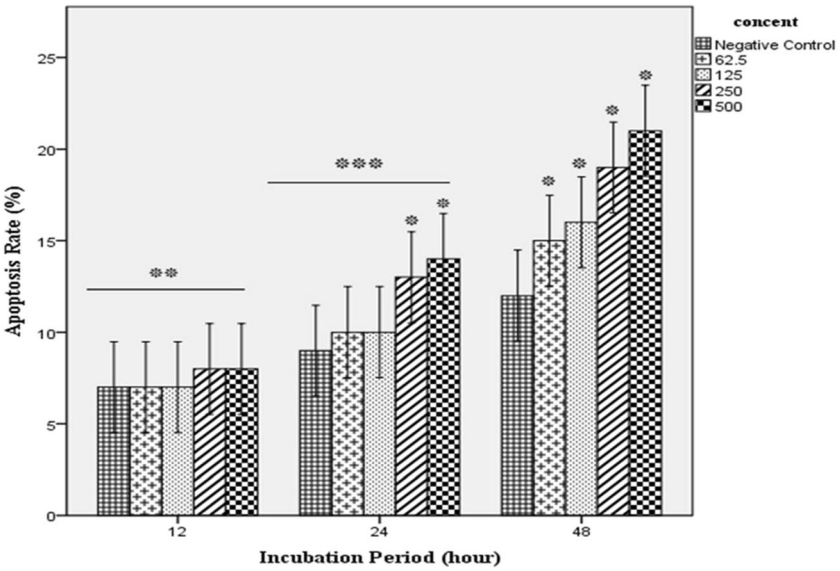

Fig. 2 Percentage of apoptosis induction relative to unexposed controls in human pulmonary epithelial cells in the presence of varying concentrations of dust particles (62.5, 125, 250, 500 µg/ml) after 12, 24, 48 h incubation times. The results are the mean ± SEM of three independent experiments. *$P < 0.05$ between varying concentration in each incubation time relative to unexposed controls and **$P < 0.05$ between 12 with 24 and 48 h incubation periods and ***$P < 0.05$ between 24 and 48 h incubation periods (One-way ANOVA followed by post hoc Turkey's test)

important cytokine that inhibits the expression and production of TNF-α in monocytes, which is dependent on the dose of IL-4 and the time-dependent affect of this cytokine on monocyte, and exerts it's affect by inhibiting transcription of the TNF-α gene [13]. So it is likely that the cause of this decrease in the production of TNF-α after an increase with the incubation time was the production of mentioned inhibitory cytokines in monocytes due to the production of TNF-α in lower incubation times. So, the first part of our study was investigating the effects of the various concentration of dust particles in different incubation time on monocytes and results showed that the production of TNF-α protein was increased by dose and time dependent manner, in which this factor can lead to apoptosis induction in epithelial lung cells by external pathway [24, 25] leading to activation of inflammatory cells and gene expression and production of inflammatory mediators in lung cells [7, 22].

In the second part, we investigated the affect of various concentrations of water-soluble particles fraction of respirable Ahvaz dust in different incubation time on apoptosis of pulmonary epithelial cells (A549). The results of the study showed that the incubation of lung epithelial cells (A549) with the particles induced increases in apoptosis compared to the control group that was not exposed to the particles by dose-dependent manner. The cause of dose-dependent manner of apoptosis is justified by this fact that dust particles by dose-dependent manner lead to defects in DNA strands and also decrease the mitochondrial membrane potential and activate caspase-9, which is an essential factor in the internal pathway of apoptosis(Upadhyay, Panduri, Ghio, & Kamp, [36]). Also,

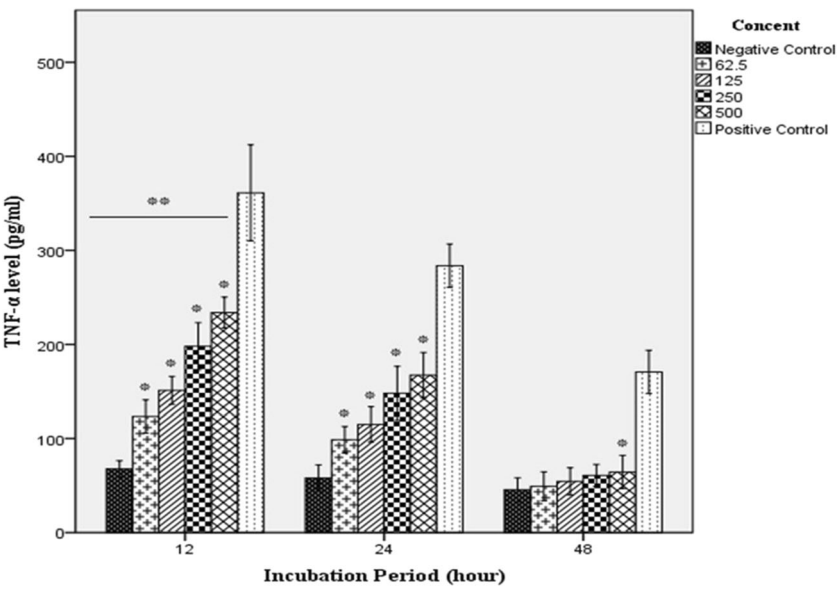

Fig. 3 Kinetics of TNF-α released from human monocytes after exposure to varying concentrations of dust particles (62.5, 12.5, 250, 500 µg/ml) in various incubation periods (12, 24, 48 h) relative to unexposed control. The results are the mean ± SEM of three independent experiments. *$P < 0.05$ between varying concentration in each incubation time relative to unexposed controls and **$P < 0.05$ between 12 and 48 h incubation periods (One-way ANOVA followed by post hoc Turkey's test)

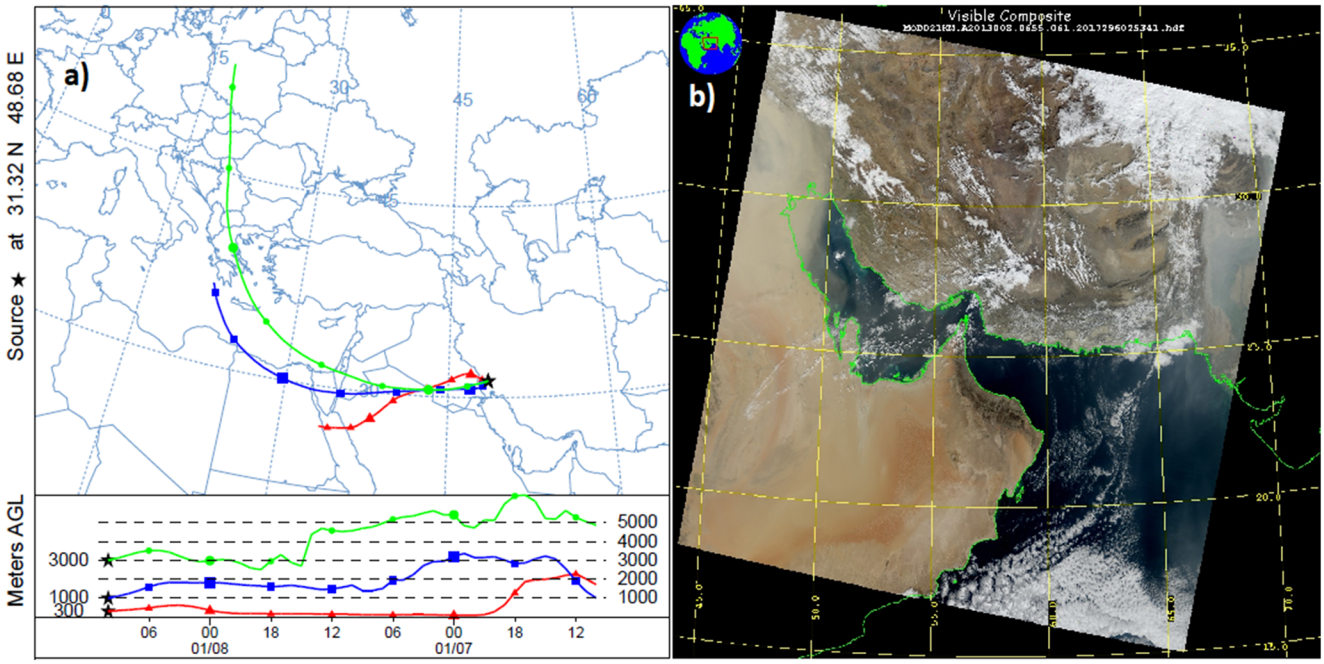

Fig. 4 48 hours backward trajectory at 10:00 AM (UTC) January 08th, 2013 (**a**) and MODIS imagery over the Middle East showing dust blowing over Ahvaz on the same day (**b**)

according to the above, the production and secretion of TNF-α can lead to activate the external pathway of apoptosis [24, 25]. According to previous studies the expression and production of TNF-α is dependent on the dose of particles [6, 38, 39]. Therefore, it can be concluded that the increases in the dose of particles led to increases in the amount of production and secretion of TNF-α, and subsequently increases the apoptosis of the cells by the external pathway. Also, in other studies, the mouse and human pulmonary epithelial cells were incubated with different concentrations of carbon monoxide and titanium dioxide, the results showed an increase in the rate of apoptosis by dose dependent manner. The cause of dose-dependent apoptosis was the dose-dependent caspase-3 activity [26, 35]. In our study, the analysis of the rate of apoptosis showed that apoptosis increases with the same concentrations of particles by increasing incubation time, which indicates that the apoptosis of lung epithelial cells was time dependent. According to similar studies, the cause of time dependence is the increase in failure in DNA strands and the reduction of double-stranded DNA by increasing the incubation time [36]. Also, another study has shown that caspase-3 activity increases with increasing the incubation time, and also 8-OHdG concentration, which is one of the most important factors in triggering the internal pathway of apoptosis, has also increased significantly with increasing incubation time. In addition, the results showed that changes in the ratio of the cytosolic fraction of cytochrome C with it's mitochondrial fraction enhanced with increasing incubation time, and the level of activity of caspase-9, which is one of the most important factors to trigger the mitochondrial apoptosis pathway, also increases with enhancing incubation time [10]. Therefore, according to the results of our research and similar studies, it can be stated that the cause of time and dose-dependent of apoptosis cells is probably the time and dose-dependent of apoptosis activity and factors involve in this process.

Conclusion

The results of our study showed that water-soluble fraction of Ahvaz air dust can increase the production of TNF-α pro-inflammatory cytokine in monocytes cells, especially when the concentration of particles in the inhalation is high, even in a short period of exposure to them, thus according to past studies, can trigger the onset of inflammation in the lungs and initiate the process of apoptosis in other pulmonary cells. On the other hand, the results showed that these particles could increase the apoptosis induction in pulmonary epithelial cells especially when the individuals exposed to a higher concentration of them in the inhalation air and during the prolonged exposure period. Therefore exposure to dust particles may possibly lead to lung damage by stimulating these two factors. Wearing facial masks and respirators is recommended to reduce the impact of dust on the MED communities particularly the west part of Iran. Alongside the physical, chemical, and biological properties of dust, we suggest evaluating spatiotemporal and geometrical characteristics of the MED storms to provide a reliable data bank to enable experts and scientists to act farther and better in the field of dust combating measures.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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