Journal of Air Pollution and Health (Summer 2018); 3(3): 135-142

Original Article

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Cytotoxicity of airborne particulate matter (PM_{10}) from dust storm and inversion conditions assessed by MTT assay

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ARTICLE INFORMATION

Article Chronology:
Received 27 July 2018
Revised 26 August 2018
Accepted 9 September 2018
Published 29 September 2018

Keywords:
Toxicological studies; Dust storm; Air pollution; MTT test; Cytotoxicity; PBMCs

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ABSTRACT:

Introduction: Airborne particles generate acute and chronic toxic effects on the human health. Cytotoxicity of air pollutants can be investigated through cytotoxicity assays. In this study, cytotoxicity of PM_{10} (particles ≤ 10 µm in diameter) from dust storm and inversion condition was compared through MTT assay on the human peripheral blood mononuclear cells (PBMCs) in vitro.

Materials and methods: PM_{10} was sampled in Tehran, Iran, 2016, in dust storm and inversion. PBMCs were isolated from the whole blood sample through Ficoll - Hypaque gradient method. Cells were treated with two suspensions of the PM_{10} from dust storm and inversion at different concentrations (50, 100, 150, 200, 250, 300, 350 and 400 µg / mL) for 24 h. Cell viability was assessed by MTT test and reported in respect to the viability in untreated cells as negative control.

Results: During the sampling period, June 6 and 12 - 15 November, 2016, were selected as the dusty and inversion days, respectively. Daily average PM_{10} in dust storm and inversion conditions were found of 220 and 345 µg / m^3, respectively. Mean of viability in the PBMCs treated by the samples from dust storm and inversion was found 85.79 ± 9.97 % and 81.58 ± 11.72%, respectively. The cell viability values were obtained between 78 - 96 % for PM_{10} related dust storm condition and 70 – 92 % for PM_{10} sampled in inversion days.

Conclusion: The results showed that the PM_{10} from dust storm as well as from inversion had the cytotoxicity effects on PBMCs. The particles related to the inversion caused toxic effects more than those from dust storm at all concentrations.

Introduction

Air pollution is a mixture of different pollutants included particulate matter (PM) and gases. PM can be originated from the anthropogenic and natural sources. Automobiles, power generators, industries, waste incinerators, biomass burning and domestic heating and cooking are the chief anthropogenic sources [1, 2]. Dust storm is one of the

Please cite this article as: Faraji M, Nabizadeh Nodehi R, Naddafi K, Pourpak Z, Alizadeh Z, Rezaei S, et al. Cytotoxicity of airborne particulate matter (PM_{10}) from dust storm and inversion conditions assessed by MTT assay. Journal of Air Pollution and Health. 2018; 3(3): 135-142

natural sources, which it has mainly participated in the air pollution of several countries in the Middle East [3, 4]. Air particles can cause toxic health effects on the human such as lung cancer and leukemia [5], allergic reactions, asthma [6], eye infections, meningitis [7, 8], mortality and hospitalization due to the cardiovascular and respiratory diseases, chronic obstructive pulmonary disease, acute myocardial infarction [9] and infertility [10]. The International Agency for Research on Cancer (IARC) has considered the outdoor air pollution and PM as the definite human carcinogen, which mainly cause the lung cancer [11].

Cytotoxicity of chemicals can be determined through *in vitro* cytotoxicity assays. Their performance is based on the several cell functions such as enzyme activity, membrane permeability and adherence of cell, production of adenosine triphosphate (ATP) and co-enzymes and the activity of nucleotide uptake. According to these functions, various methods are used for measuring the cytotoxicity such as crystal violet, colony formation, tritium-labeled thymidine uptake and colorimetric method by using tetrazolium dye, briefly MTT. Among mentioned methods, MTT is safe, easy-to-use, reliable with a high reproducibility. In MTT assay, NAD(P)H-dependent cellular oxidoreductase enzymes in the active cells reduce the tetrazolium dye MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to the intracellular purple formazan [12].

MTT method was studied to assay cytotoxicity of $PM_{2.5}$ (particles ≤ 2.5 μm in diameter) and $PM_{2.5-10}$ (particles between 2.5-10 μm in diameter) ambient air pollutants [13], dust storm $PM_{2.5}$ [14], urban PM_{10} and $PM_{2.5}$ [15], Fine particulate matter (PM_1 and $PM_{2.5}$) in the Milan urban area [16], ambient $PM_{2.5}$ [17].

To the best of our knowledge, toxicity of PM_{10} (particles ≤ 10 μm in diameter) from dust storm and inversion sources have not yet been compared by MTT method. So, this study aimed to compare cytotoxicity of PM_{10} collected in dust storm and inversion conditions through MTT assay on the human peripheral blood mononuclear cells (PBMCs).

Materials and methods

The study stages, including sampling and preparation of PM_{10}, PBMCs isolation, cell treatment with the particle suspension, MTT assay and statistical analysis, are visualized in Fig. 1. Chemical analysis on the samples has been reported in other study [3].

Air sampling

Particles were collected in Tehran, Iran (35˚70′66.00″N, 51˚39′38.55″E) (Fig. 2). Since, dust storm and inversion occur in Tehran mainly in spring and autumn, respectively, 24 h sampling was done over the two periods, from April 26 to June 7 and September 24 to November 15, 2016.

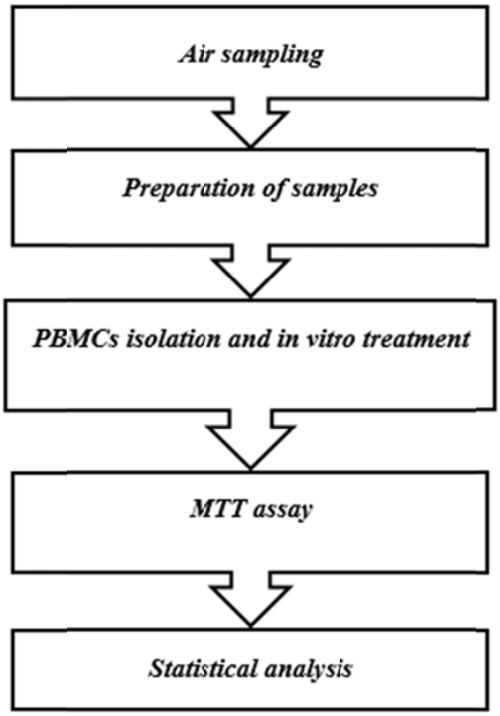

Fig. 1. Study stages

Particles were sampled using a high-volume sampler (1.3 – 1.7 m^3 / min) (Grasebey, USA) equipped with fiberglass filter (8×10 inch, grade G 653 Whatman, USA). Filters were weighed with an analytical balance (± 10 mg) before and after sampling to calculate the mass of sampled PM. The sampler was installed based on the United States Environmental Protection Agency (USEPA) instruction [18] at the height of 10 m above the ground level far away from any obstruction to prevent the potential effects of natural and anthropogenic obstacles on the air flow and PM concentration.

Preparation of PM_{10} samples

In the first stage of PM_{10} preparation, two conditions of dust storm and inversion must be differentiated. Dust storm and inversion conditions were distinguished according to the Hoffmann's criteria [19] (Table 1) and the report of the Tehran Air Quality Control Company (TAQCC), respectively. The particles were extracted from the filters through the dry ultrasonic (Elma-ultrasonic, Germany) followed by sweeping with a smooth brush [3]. The extracted PM_{10} was weighed and stored into the endotoxin - free vials at -18°C until their use in biological test. Since, the fiberglass fibers have toxicity effects on cells [15], their presence was inspected in the extracted samples by using a scanning electron microscope (SEM) (HITACHI, SU3500, Japan)

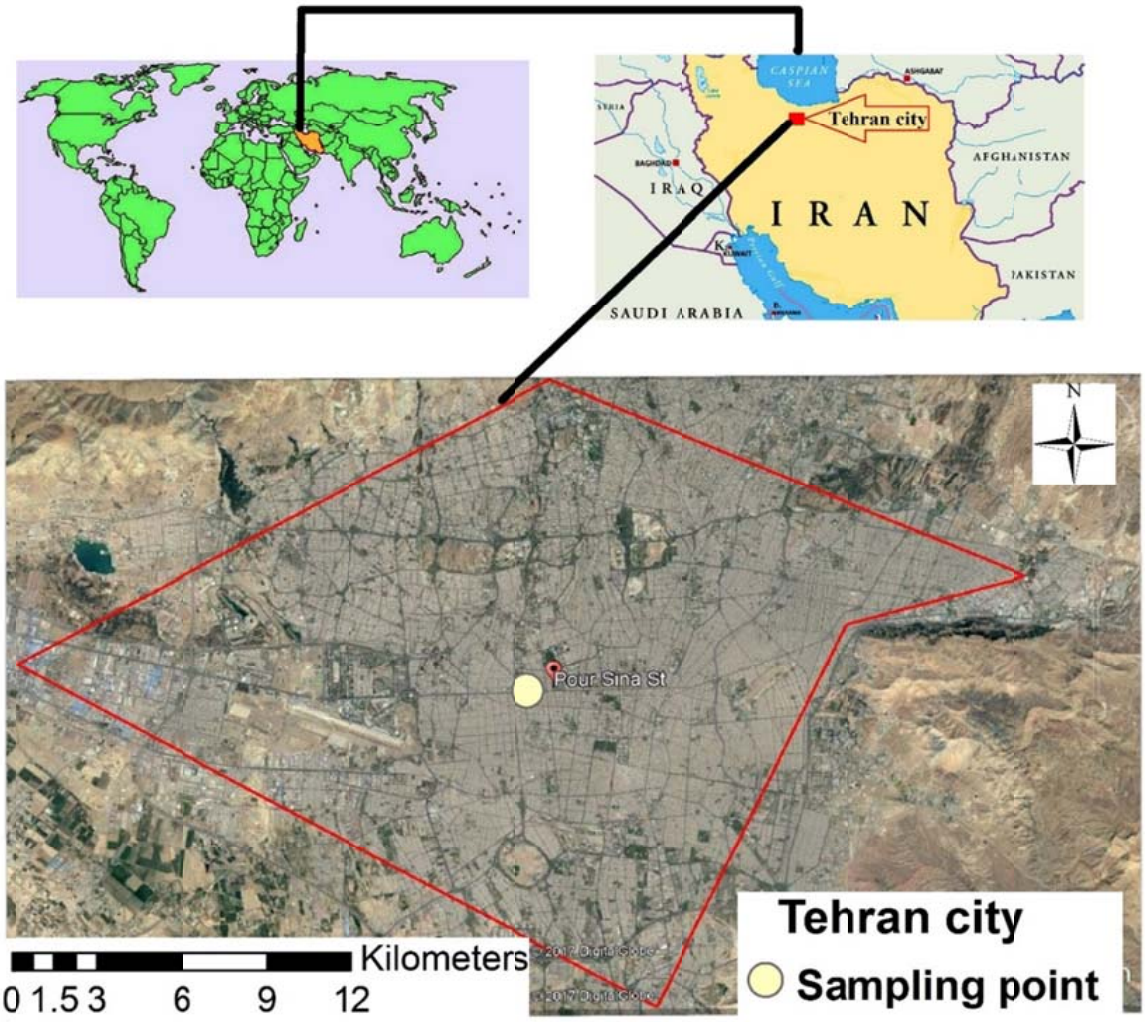

Fig. 2. Map of study area and PM_{10} sampling station

Table 1. Dust storm classification

Category	Visibility (m)	Wind speed (m / s)	PM_{10} (µg / m^3.h)
Dusty Air (DA)	Haze	-	50-200
Light Dust Storm (DS1)	< 2000	-	200 - 500
Dust Storm (DS2)	< 1000	> 17	500 - 2000
Strong Dust Storm (DS3)	< 200	> 20	2000 - 5000
Serious Strong DS (DS4)	< 50	> 25	> 5000

PBMCs isolation and in vitro treatment

The volume of 20 mL whole blood sample was collected from the healthy volunteer and put into the heparinized tube as the anticoagulant and processed within 2 h. The method of Ficoll-Hypaque gradient was used to isolate the PBMCs. Briefly, 40 mL Ca^{2+}/Mg^{2+} -free PBS (Biosera, France) was added to whole blood sample in the laminar flux hood to dilute it. Cells were isolated from the diluted blood with addition of 30 mL Ficoll-Hypaque solution (Biosera, France) by density centrifugation (22 min, 2000 rpm, no acceleration, no brake). Then, layer of PBMCs was collected and washed by the lysis buffer and isolation buffer and isolated through centrifugation (400 g, 14 min, acceleration 6, brake 4). The number of 200,000 cells were seeded in each well of a 96-well plate in 100 mL complete RPMI-1640 culture medium (Gibco BRL, San Diego, CA) and cultured in a humidified incubator at 37 ºC with 5% (v/v) CO_2.

Two suspensions from dust storm and inversion were separately prepared in the culture medium. The cultured cells were treated with mentioned suspensions at different concentrations (50, 100, 150, 200, 250, 300, 350 and 400 µg / mL) and incubated at 37 °C for 24 h. Experiments at six concentrations were done in triplicate.

MTT assay

MTT solution in the final concentration of 0.5 mg / mL (Sigma Chemical Company, St. Louis, MO, USA) was added to the wells and incubated for 4 h. Insoluble formazan crystals generated as the byproduct of MTT assay were dissolved in 150 µL dimethylsulfoxide (DMSO) (Sigma Chemical Company, St. Louis, MO, USA). Then, the absorbance of samples was measured using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at 570 nm. Finally, cell viability percent was calculated using Eq. (1):

$$Cell\ viability(\%) = (A_S - A_b)/(A_{NC} - A_b) \times 100 \quad (1)$$

where A is the absorbance, b is the blank, and S and NC are the sample and negative control (untreated cells), respectively [20].

Statistical analysis

The data were analyzed with Excel 2016 software and reported as means ± standard deviation of three independent experiments.

Results and discussion

PM_{10} concentration

According to the considered criteria, June 6 and November 12-15, 2016, had respectively the condition of dust storm and inversion over the particle sampling period. Particle sampling information at the two conditions is described in Table 2.

Particle extraction

Morphological structure of blank filter and extracted sample were shown in Fig. 3a and 3b, respectively.

Table 2. Information on the PM_{10} sampling during dust storm and inversion conditions

Condition	Date	PM_{10} mass (g)	Air volume (m^3)	Daily average PM_{10} (µg / m^3)
Dust storm	June 6	0.57	1634.32	348.40
Inversion	November 12	0.48	2028.32	236.60
	November 13	0.41	2051.22	201.20
	November 14	0.43	2031.98	211.32
	November 15	0.43	1840.35	233.05

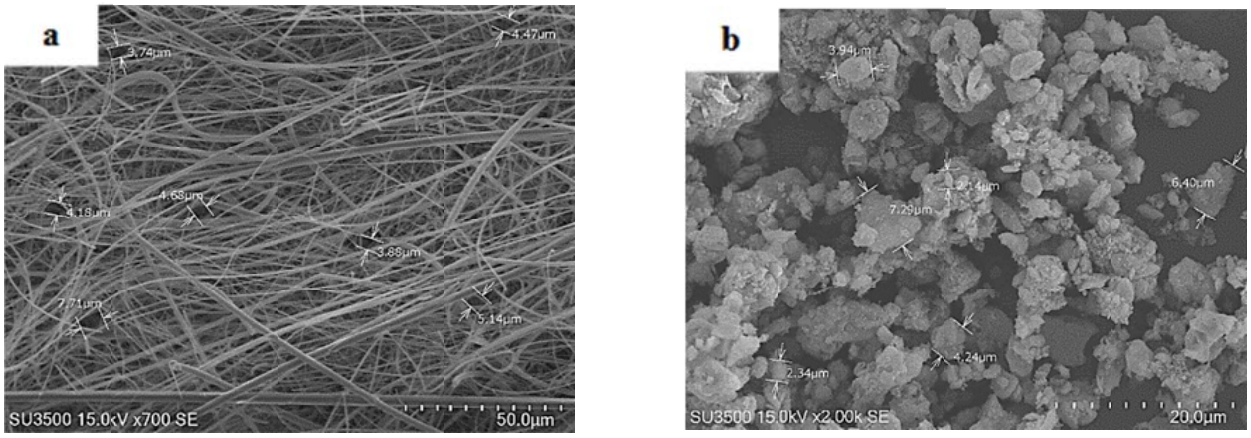

Fig. 3. Morphological structure of blank filter (a) and extracted sample (b)

Table 3. Viability percent of peripheral blood mononuclear cells (PBMCs) in MTT assay treated by PM_{10} from dust storm and inversion at concentrations of 50 - 400 **µg** / mL.

	PM_{10} concentration (µg / ml)								Total
	50	100	150	200	250	300	350	400	
	%Cell viability related to dust storm PM_{10}								
Mean	96.00	93.33	92.33	90.33	87.33	83.00	78.33	65.67	85.79
SD*	4.36	5.69	5.51	10.02	8.62	5.29	3.21	5.03	9.97
	% Cell viability related to inversion PM_{10}								
Mean	92.00	90.00	91.00	87.00	84.33	79.33	70.33	58.67	81.58
SD*	4.36	4.00	6.56	3.00	5.03	4.04	5.51	3.21	11.72

*: Standard deviation

Cytotoxicity analysis

Mean of viability for PBMCs treated by the samples from dust storm and inversion was found 85.79 ± 9.97 % and 81.58 ± 11.72 %, respectively. Viability percent of PBMCs at concentrations of 50 - 400 µg / mL represents in Table 3.

The dusty day with daily average PM_{10} concentration of 348.40 µg / m^3 could be categorized in the light dust storm class based on the Hoffmann classification in Table 1. Also, the world meteorological organization (WMO) classified dust events according to visibility into the four class-

es: (1) dust – in - suspension: visibility usually less than 10 km; (2) blowing dust: visibility 1-10 km; (3) dust storm: visibility 200 - 1000 m; and (4) severe dust storm: visibility less than 200 m [21]. In the current study, the dust storm day with the visibility of 5.95 km (Table 2) could be classified in the dust-in-suspension or blowing dust class. At both the conditions, daily average PM_{10} concentration was up to 6.96 times higher for the dust storm condition and 4.4 times higher for the inversion, as compared to that with the national guideline (50 µg / m^3).

Based on the comparison between the morphological structure of blank filter in Fig. 3a and the extracted particles in Fig. 3b, it can be concluded that fibers were not observed in the extracted particles. As a result, the observed toxicity effects in MTT assay could be certainly assigned to the particles.

According to Table 3, it can be concluded that both types of particles had cytotoxicity effects on the PBMCs and they could suppress cell activity. The particles related to the inversion caused toxic effects more than those from dust storm at all concentrations (mean of viability of 85.79 ± 9.97 % in dust storm *vs*. 81.58 ± 11.72 % for inversion). The cell viability values were obtained between 78 – 96 % for PM_{10} related dust storm condition and 70 – 92 % for PM_{10} sampled from inversion. Cell viability was decreased less than 80 % at concentration of 350 µg / mL for the both types of particles (78 % for the PM from dust storm and 70 % for it from inversion). This reduced cell viability could be associated to the different physiochemical characteristics of particles in dust storm condition and inversion [18, 22]. Particles from dust storm and inversion had dissimilar nature because they release from different sources. Particles related to dust storm condition mainly disseminate from natural sources. Against, particles allocated to inversion in Tehran release from anthropogenic sources mainly fossil fuels and vehicles [18]. In comparison between the chemical compositions, frequent ionic components were found NO_3^-, Cl^-, SO_4^{-2} and Ca^{+2} during dust storm (72.18 % of PM mass) and SO_4^{-2}, NO_3^-, NH_4^+ and Cl^- during inversion (87.78 % of PM mass). For both conditions, Si, Fe and Al were identified as the dominant elements respectively in dust storm condition 96.62 % of PM mass and in inversion 59.16 % of PM mass [3]. Usually, the particulates resulted from the anthropogenic sources have the higher concentrations of pollutants such as heavy metals and polyaromatic hydrocarbons [11].

Conclusion

In the present study, toxicology effect of PM_{10} was investigated on the PBMCs by MTT method. This effect was compared between particles from natural and anthropogenic sources, respectively dust storm and inversion. Results were verified that both category of the particles could decrease cell viability. But, particles from inversion condition generate the toxic effects more than those from dust storm. Different percentages of viability in the treated cells can be due to diverse chemical composition and physicochemical properties of the particles in studied conditions.

Financial supports

This study was financially supported by the Institute for Environmental Research (IER) of Tehran University of Medical Sciences (TUMS) (grant number 95-03-46-32844) and Iran National Science Foundation (INSF) (grant number 95831261).

Competing interests

All of the authors declare that they have no actual or potential personal or financial competing interests.

Acknowledgements

The authors would like to thank the IER and INSF for financially supports. Also, they are grateful to the Immunology, Asthma and Allergy Institute (IAARI) for technically support and staff collaboration.

Ethical considerations

This study was permitted by the ethics committee of the Tehran University of Medical Sciences. Written informed consent was obtained from volunteer before starting the study. Also, the results of the research will be published with the permission of the funders. Also, all authors agree to submit their manuscript to JAPH. The authors confirm that the manuscript have not been submitted or published elsewhere in any language.

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