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Effects of inhaled particulate matter ($PM_{2.5}$) on uterine quality in female rats as an animal model

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ABSTRACT

Air pollution plays an important role in the health of reproduction. Studies on the destructive effects of environmental pollutants have been focused more on their effects on testicular and sperm quality, while the study on the female reproductive system has received less attention. Therefore, in this study, the effects of $PM_{2.5}$ on the female mice's uterine quality were assessed. A total of 24 Wistar albino rats were divided into three treatments and control for a three months' period. Three groups were defined including healthy control (C) that used air with a clean standard condition, Treatment 1 (T_1) was exposed to only gaseous pollutants, while treatment 2 (T_2) to gaseous pollutants plus PM2.5. Uterus tissue was removed after dissection of rats, then the oestrogen and progesterone receptors, immunohistochemical assays, and oxidative stress activity for all groups were evaluated. The activity of enzymes involved in oxidative stress (MDA, SOD, GPx, and CAT) in the treatment and control groups were examined in mice uterus tissue after three months. T_1 and T_2 showed a statistically significant decrease in SOD, GPx, and CAT compared to control. On the other hand, T_2 exhibited a significant decline in these enzymes compared to T_1. In the case of MDA, T_1 and T_2 displayed a significant elevation compared to control ($p < 0.01$ and $p < 0.001$) respectively, while T_2 indicated a significant decrease compared to T_1 ($p < 0.01$). In T_2, the estrogen and progesterone receptors (ER and PR) Immunohistochemical results showed a sharp drop compared to control and T_1. Serious programs should be implemented to control and reduce PM2.5 emissions in metropolitan areas. These findings may be useful to provide insights for reducing infertility disorders caused by exposure to PM2.5 or other air pollutants.

Keywords: Immunohistochemical assay, $PM_{2.5}$, Pollution, Stress oxidative, Uterus.
Article type: Research Article.

INTRODUCTION

Air pollution is one of the major problems facing human beings and in some cities. It is becoming more severe due to the increased industrial activities, consumption of fossil fuels, and population density (Cohen *et al*. 2017). The lives of 87% of people are endangered due to urban pollution. Air pollution is caused by solid and liquid particles and certain gases that are suspended in the air. These particles and gases can come from the exhaust of cars and trucks, factories, dust, pollen, mold spores, volcanoes, heavy metals and forest fires. Their teratogenic effects have also been reported in different studies (Mehrandish *et al*. 2019; Yamin *et al*. 2020; Usunobun *et al*. 2022; Rao *et al*. 2022). Air pollution has adverse effects on the health of people in the community and leads to premature death, cardiovascular disease, bronchitis, respiratory disorders, and cancer (Lelieveld *et al*. 2017). Air

pollution is the presence and spread of one or more pollutants, including solid, liquid, gas, radioactive and non-radioactive radiation in the open air to an extent and for a time that alters its quality in a way that is harmful to humans and other living organisms or plants (Fallah Jokandan 2016). Particulate matter that can be inhaled varies in chemical composition, chemical properties, degradation time, and ability to propagate over long or short distances. These particles cross the body's natural defense barrier and penetrate deep into the processes, exacerbating asthma and impaired lung function. If the suspended particles concentrations exceed the standard limits, may cause death and are divided into the following types. Particles in air pollution include coarse atmospheric particles with an aerodynamic diameter between 2.5 and 10 μm (PM_{10}), fine particles less than 2.5 μm in diameter ($PM_{2.5}$), and ultrafine particles less than 0.1 μm in diameter ($PM_{0.1}$). Numerous studies have shown the destructive and pathogenic effects of smaller particles, higher and more severe than larger particles (Münzel *et al*. 2018). In scientific classification, in which air pollutants are known as standard pollutants, six types of pollutants have been identified, including ozone, particulate matter (PM: Particulate Matter), nitrogen dioxide, sulfur dioxide, lead, and carbon monoxide (Cherkasov *et al*. 2014; Seinfeld *et al*. 2016; Asadifard & Masoudi 2018; Masoudi *et al*. 2019).

Respiratory diseases, cancers, and infertility are some of the diseases that threaten the health of society more than others due to their unhealthy lifestyle (Moghaddam *et al*. 2016; Moghaddam *et al*. 2020). The PM_{10}, and $PM_{2.5}$ which contain heavy metals and PAHs, can have adverse effects on the human reproductive system by altering the sex hormone levels of estrogen and disrupt the functioning of this system (Veras *et al*. 2010; Sharma *et al*. 2013). Loss of physiological balance between oxidants and antioxidants in the body following exposure to air pollutants causes oxidative production reactions and cell damage. This phenomenon can change the structure of DNA, proteins, and cell membranes. These factors also reduce the ability of blood vessels to dilate and increase blood pressure during pregnancy (Arbak *et al*. 2004). The most vulnerable period to exposure to air pollution is in pregnancy and the foetus, during which embryonic and organ development occurs and the foetus lacks immune function during this period (Thornton *et al*. 2013). Exposure to $PM_{2.5}$ increases pregnancy complications such as intrauterine growth retardation, preterm delivery, and low birth weight due to the increased production of pro-inflammatory stickiness and prostaglandins (Dadvand *et al*. 2013). The oestrous cycle is a repetitive process in non-primordial vertebrates and is equivalent to the menstrual cycle in primates, which indicates a change in hormone levels due to ovarian activity under the influence of pituitary hormones and causes changes in the ovaries, uterus, and vagina. There are four phases: proestrus, estrus, metestrus, and diestrus, in which the vaginal mucosa undergoes extensive structural changes under the influence of FSH, LH, progesterone, and estradiol (Gronemeyer 1991). Estrogen is a group of the most important female sex hormones, which are mainly produced in the ovaries. In the normal cycle of the ovary, estrogen is released from the egg in the follicular stage. By binding to the estrogen receptor in the cytoplasm, estrogen increases the production of DNA, RNA, and other proteins in the target tissue. Estrogen in the uterine wall causes the endometrium to grow and become hyperemic. Also, in the hypothalamus, the release of sex-stimulating hormone in the glands decreases under the influence of estrogen, and in the pituitary gland, the release of FSH and LH decreases. Steroid hormone receptors are intracellular, DNA-binding proteins that act as regulators of cell growth and development. Hormone binding causes the receptor to deform, followed by receptor-hormone binding to the nucleus. At the nucleus of the complex, it binds to specific sequences of nucleotides that regulate the transcription of target genes (Shick *et al*. 1995). The presence of the estrogen receptor gene does not mean that all cells express it. Due to estrogen binding, this receptor is activated and expresses a number of its target genes that are also involved in cell division, i.e., they act as a transcription factor, so not all of these receptors are expressed. Since cell division increases dramatically, only a fraction of them are expressed that can respond to estrogen. These genes include those associated with adhesion molecules, growth factor cytokines, degrading enzymes, and extracellular matrix components (Parker *et al*. 1991). Progesterone is released from the corpus luteum during the normal ovulation cycle. This hormone is also released during pregnancy. The progesterone receptor activates its target genes by binding to the hormone as a transcription factor. Also known as "NR3C3", it is an intracellular receptor activated by the steroid hormone, progesterone. This protein receptor in humans is encoded by the PGR gene, located on the long arm of chromosome 11 (Misrahi *et al*. 1987). In the cell, there is a dependence between ER and PR in the absence of estrogen. An elevation in the number of ERs and a deline in the number of PRs can be expected, or vice versa, and may affect the amount of hormone in the tissue of the number of receptors (Whitaker *et al*. 1991). It is important to know the exact and effective mechanism of contaminant particles on the tissue structure of the ovary and uterus to find effective prevention or treatment for

injuries, as well as measuring changes in ER and PR receptors, which is an indicator in determining cancer including breast and uterus cancers (Gronemeyer *et al*. 1991). Although the health of men and women is equally important in reproduction, studies on the destructive effects of environmental pollutants have been higher on testicular and sperm quality, while the study of the female reproductive system has received less attention. Therefore, in this study, the effects of $PM_{2.5}$ on female mice uterine quality were studied.

MATERIALS AND METHODS

Chemicals and Devices

Chloroform from Merck (Germany) and immunohistochemistry kit Dako from (Denmark) were purchased. The Zelbio kit (Germany) was used to study changes in oxidative stress enzymes. Millipore PTFE filters (Hesperia, CA, USA), Gas Chromatography-Mass Spectrometry (Agilent 5890A model), vacuum pump (Model LFS-113; Gilian Instrument Corp), HEPA filter model H13 (Camfil-FARR, Switzerland) were used.

$PM_{2.5}$ Preparation

Sampling location

One of the known sources of pollution was Shahid Beheshti University in Tehran, Iran. Therefore, sampling was performed on the roof of Bam Public Health School (SPH) belonging to this university (35.7991º N, 51.3947º E). Samples were collected at a height of 20 meters from the ground.

Digestion and sampling $PM_{2.5}$

The $PM_{2.5}$ sampling was done by Echo PM Low volume sampler in ambient air next to the animal room pilot (EPA 2017a). In order to collect $PM_{2.5}$, sampling filter were used. Filters were quartz microfiber with 47 mm in diameter, Whatman International Ltd; 20 L/min flow rate with 24 h (9.00 AM - 9.00 AM). Before sampling, quartz microfiber filters were washed with sterile distilled water and placed in an oven at 105 °C for 2 h (Helzer *et al*. 2019). The QM filters were then subjected to a relative humidity of 40-50 at room temperature equivalent to 18-20 ° C for 24 hours (Helzer *et al*. 2019). In the last step, to prevent evaporation and optical decomposition, the filters were placed in aluminum foil and kept at a temperature of -10 ºC (Mills *et al*. 2009).

Digestion and analysis of metals procedure

A quarter of each QM filter was isolated and crushed in a 15-mL Teflon container. Next to the Teflon dish, 2.5 mL HNO_3 (69%) and 2.5 mL concentrated $HClO_4$ (70%) were added and then stored at 170 °C for 4 h. A hot plate with a temperature of 95-100 °C was used to dry the solution completely. Subsequently, 2.5 mL HNO_3 and 2.5 mL distilled water were added to the samples and the samples were shaken at 180 rpm for 30 min (Helzer *et al*. 2019). The resulting volume was filtered through a Whatman filter (Paper No. 42; 125 mm), then 10 mL of the diluted solution was placed in a plastic vial at 4 °C and stored for further analyses. A clean filter was used as a control and these steps were repeated (Helzer *et al*. 2019). ICP/MS (Model 7900, CA) was used to analyze metal concentrations.

Digestion and analysis of PAHs procedure

A quarter of each QM filter was crushed in a 15-mL Teflon container. Then 2.5 mL CHOH and 2.5 mL of CH_2Cl_2 were added to the Teflon dish and sonicated in an ultrasonic bath at 20 kHz for half an hour (Elmasonic S 80H). Finally, the obtained solutions were filtered through 0.22-mm Millipore PTFE filters and the 16-PAH concentration was evaluated using Gas Chromatography-Mass Spectrometry. As mentioned before, a clean filter was used as a control filter and all steps were performed.

In vivo study

Experimental animals and ethical aspects

Wistar rats care, and the experimental steps were performed in full accordance with the criteria of care and use of institutional animals Medical Sciences Ethics Committee of Shahid Beheshti University, Tehran, Iran (Ethical code: IR.1398.054). In this study, twenty-four Wistar rats (85 ±10 g in weight, 4 weeks) were purchased from Pasteur Institute, Tehran, Iran. The number of rats was minimized as far as possible. Wistar rats were housed separately in cages with free access to water and food, under light / dark cycles (12 L:12 D), constant temperature

(24 ± 22 °C), and humidity (57%). After 7 days of acclimatization, the rats were randomly divided into three groups (n = 7 per group).

Design, exposure construction and control rooms

Exposure and control chamber (1 m in length, 1 m in width and 1.5 m in height) made up of MDF with a roof made of glass. Three different exposure groups in three chambers were designed in animal pilot room (**Fig. 1**).

Group 1: Health Control group (C); which was included air with standard condition. In the control group ambient air was introduced into the chamber with 12-L min^{-1} flow rate via vacuum pump with HEPA filter model H13 and active carbon air filter.

Group 2: Treatment 1 (T_1); which was only included gaseous pollutants. Ambient air was entered into the chamber number 2 with 12-L min^{-1} flow rate via a vacuum pump. To eliminate PM pollutant, a HEPA filter model H13 was placed in the entrance of vacuum pump. Accordingly, T_2 contained gaseous pollutant alone.

Group 3: Treatment 2 (T_2); included $PM_{2.5}$ and gaseous pollutants. In chamber 1, ambient air with 12 L min^{-1} flow rate was passed into the chamber through low volume sampler (LVS), PM (TCR Tecora, Italy). However, sampling filter was removed because LVS is an instrument used to collect samples of $PM_{2.5}$ and PM_{10}. Without the sampling filter, ambient air that enters in the chamber will include PM. In this study, the $PM_{2.5}$ was used. Therefore, ambient air in Chamber 1 was included $PM_{2.5}$ and gaseous pollutants.

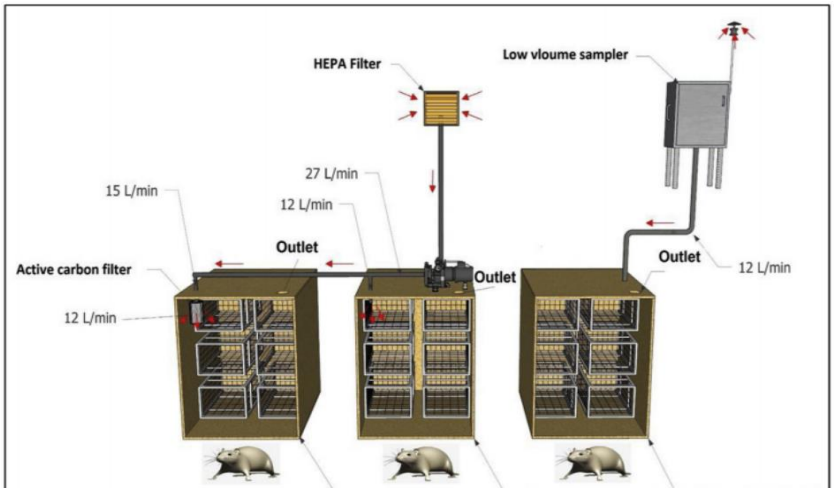


Fig. 1. Schematic of exposure method.

Animal study design

In this study, the SO_2, O_3 and NO_2 concentrations in the animal chamber were examined. The $PM_{2.5}$ and gaseous pollutants concentrations were measured via beta attenuation monitoring and ultraviolet (UV) fluorescence during exposure time (5 h: 9.00 AM to 2.00 PM) respectively. The three-month treatment period was designed. Wistar rats were employed for five hours per day (9.00 am to 2.00 pm), four days a week.

Oxidative stress analyses

The uterus tissue removed from mice were lysed then superoxide dismutase (SOD), malondialdehyde (MDA), glutathione Peroxidase (GPx), and catalase (CAT) activity was evaluated in tissue samples lysate using the reagent kit according to the manufacturer's instructions.

Immunohistochemistry (IHC)

At the end of the three-month treatment period, the uterus tissue of mice in each group was dissected. A portion of tissue was fixed in 10% neutral buffered formalin solution for 24 h. The fixed tissues were processed routinely and were then embedded in paraffin and sectioned to 5 mm thicknesses for immunohistochemistry analysis. To perform the estrogen and progesterone receptors (ER and PR) immunohistochemistry (IHC) techniques, the samples first treated with antibodies. They were incubated using a secondary antibody using a two-stage immunohistochemistry detection kit. All sections stained were studied under a light microscope at 40 magnifications. According to the results reported in Table 1, ER and PR expressions were evaluated.

Statistical analysis

Data were reported as mean ± SD and the graphs were plotted using GraphPad Prism version 8. Data were analysed using ANOVA test followed by post-Tukey post hoc test and a p-value less than 0.05 was considered as a significant difference.

Table 1. The scoring amount of ER and PR immunohistochemistry expression of uterine tissue.

	Indicator	Point	Control	Treatment 1	Treatment 2
	Lack of expression in the nucleus of epithelial cells and endometrium	0			
ER Expression	Expression in less than 25% of cells	1	3	2	2
	Expression in 25-25% of cells	2			
	Expression in more than 50% of cells	3			
	Lack of expression in the nucleus of epithelial cells and endometrium	0			
PR Expression	Expression in less than 25% of cells	1	3	2	2
	Expression in 25-25% of cells	2			
	Expression in more than 50% of cells	3			

RESULTS

The $PM_{2.5}$, O_3, NO_2, and SO_2 concentration

The concentrations of $PM_{2.5}$ in the three months of exposure were showed in Table 2. The mean concentration of $PM_{2.5}$ in the three-month exposure was 41.76 ± 4.42 µg m^3. The concentration of $PM_{2.5}$ in the 40-days exposure was higher than the WHO guideline 25 µg m^3, ($p < 0.05$) (Lebeau *et al*. 2008). The mean concentration of O_3 was 8.76 ±1.05 ppb and lower than the WHO guideline (100 ppb; $p < 0.05$) (Helzer *et al*. 2019). The mean concentration of SO_2 was 3.76 ± 1.22 ppb and lower than the WHO guideline (20 ppb; $p < 0.05$) (Helzer *et al*. 2019). The mean concentration of NO_2 was (59.87 ±7.02 ppb and lower than the WHO guideline (100 ppb; $p < 0.05$) (Helzer *et al*. 2019).

Table 2. Concentration of $PM_{2.5}$, O_3, SO_2, and NO_2 in ambient air of pilot animal room.

Contaminants	Duration of Exposure	Unit	Mean	Range	WHO guideline
$PM_{2.5}$	3 Months	µg m^{-3}	41.76± 4.42	11.12- 54.29	25
O_3	3 Months	ppb	8.76± 1.05	10.00- 20.00	100
SO_2	3 Months	ppb	3.76± 1.22	4.00- 8.00	20
NO_2	3 Months	ppb	59.87± 7.02	41.00- 74.00	100

Concentration of heavy metals bound $PM_{2.5}$

Table 3 shows the concentrations of heavy metals bound to $PM_{2.5}$. The results reported heavy metal concentration as Al> Ca> Na> Cu> Cd> Cr> Ni> Pb> Fe> Mn, respectively. Aluminum exhibited a highest level, while magnesium a lower concentration after three months of treatment.

Table 3. Concentrations of heavy metals bound $PM_{2.5}$ in the exposure duration (mg m^{-3}).

Heavy metals	40 Days
Al	87.10± 6.10
Ca	61.45± 1.61
Cd	2.10± 3.00
Cr	0.42± 0.10
Cu	1.40± 4.01
Fe	0.10± 0.10
Mn	0.15± 0.09
Na	4.00± 2.42
Ni	0.29± 0.20
Pb	0.27± 0.11

Concentrations of PAHs bound $PM_{2.5}$

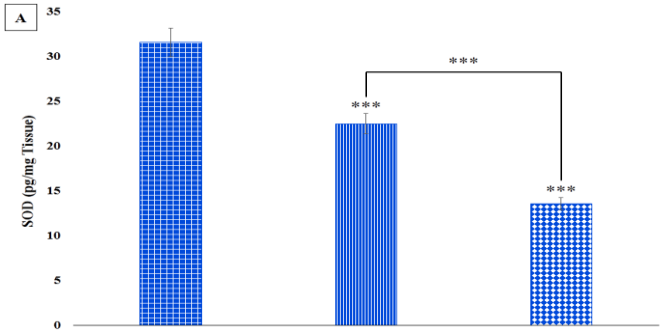
The concentrations of PAHs bound with $PM_{2.5}$ were showed in Table 4. The sum concentration of 16-PAHs bound $PM_{2.5}$ in the three months exposure was 40.59 ± 10.00 ng m^3. The order of 16-PAHs was Phenanthrene> Naphtalene > Anthracene > Chrysene > B(a)P > Acenaphten > Acenaphtylen > Pyrene > Fluorantene > Florene > Benzo(a)ant > B(k)F~Indeno (1,2,3-cd)Pyrene ~ B(b)F ~ Dibenzo(a,h)Anthracene ~ Benzo g,h,iPerylene. According to findings, the concentration of Phenanthrene was higher than other PAHs bound $PM_{2.5}$ that were inhalated by rats after three months exposure.

Table 4. Concentration of 16-PAHs bound $PM_{2.5}$ in two exposure periods (ng m^{-3}).

16-PAHs	Three Months
Naphtalene	7.1 2± 15.33
Acenaphtylen	1.32 ± 0.5
Acenaphten	1.99 ± 1.54
Florene	0.78 ± 0.45
Phenanthrene	17.66 ± 9.07
Anthracene	4.32 ± 3.11
Fluorantene	1 ± 0.10
Pyrene	1.11 ± 0.23
Benzoant	0.30 ± 0.10
Chrysene	2.99 ± 0.17
B(*b*) F	ND
B(*k*) F	ND
B(*a*) P	2.00±1.88
Dibenzo(a,h) Anthracene	ND
Benzo(g,h,i) Perylene	ND
Indeno(1,2,3-cd) Pyrene	ND
Sum	40.59 ±10.00

Oxidative stress

The activity of enzymes involved in oxidative stress, i.e., MDA, SOD, GPx, and Catalase in the treatment and control groups were examined in the mice uterus tissue after three months. T_1 and T_2 showed a significant decrease in SOD enzyme compared to control ($p < 0.001$). However, in comparing between the two treatments, T_2 exhibited a significant decline compared to T_1 ($p < 0.001$; Fig. 2A). In the case of the activity of MDA enzyme, T_1 and T_2 displayed a significant elevation compared to control ($p < 0.01$, and $p < 0.001$ respectively). On the other hand, once comparing between the two treatments, T_2 revealed a significant decline compared to T_1 ($p <0.01$; Fig. 2B). In the case of the activity of GPx enzyme, T_1 and T_2 exhibited a significant drop compared to control ($p < 0.01$ and $p < 0.001$ respectively). In addition, a significant decrease was observed in T_2 compared to T_1 ($p < 0.05$; Fig. 2C). There was also a significant decline in the catalase enzyme in the two treatments (T_1 and T_2) compared to control ($p < 0.05$, and $p < 0.01$). Moreover, a significant decrease was observed in T_2 compared to T_1 ($p < 0.05$; Fig. 2D).



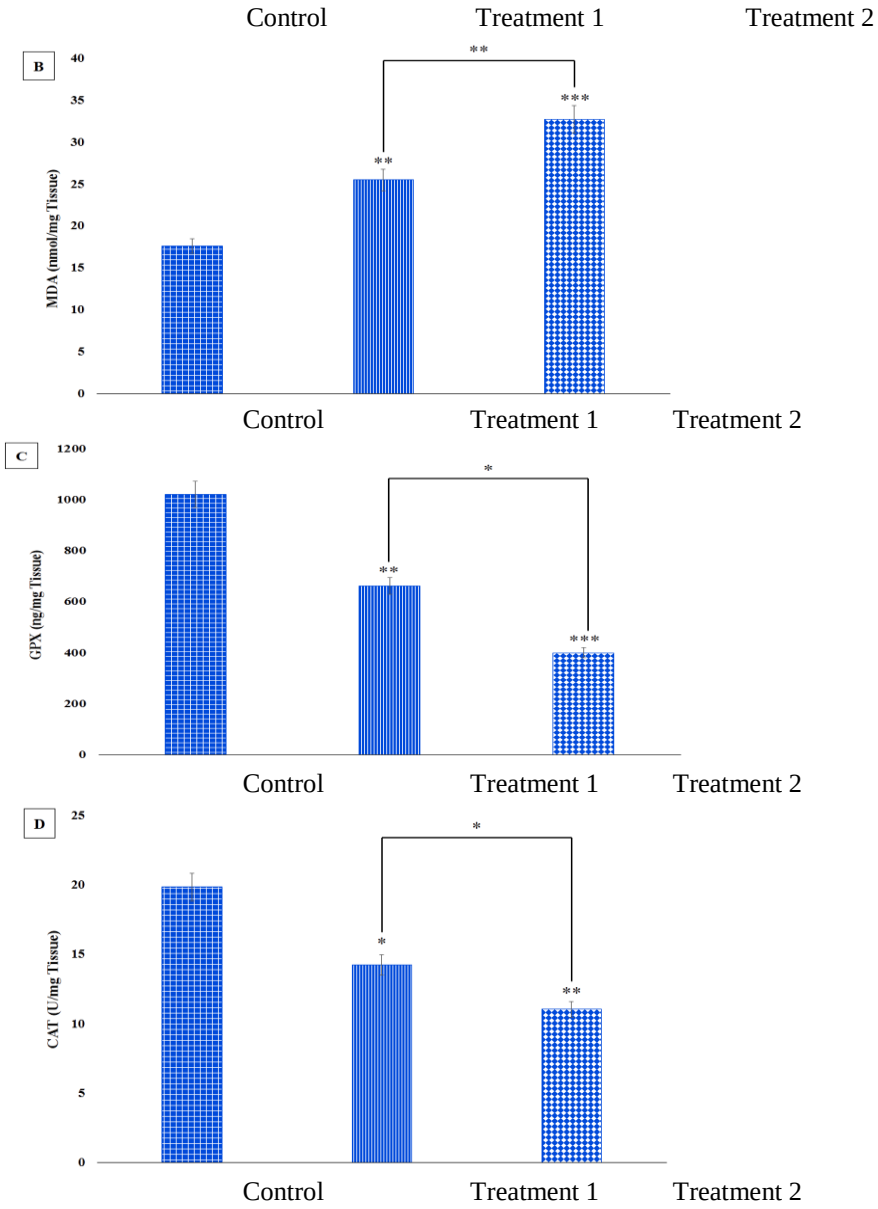


Fig. 2. Comparison of the oxidative stress indices in control and treatments in Wistar mice. Mean and standard deviation (Mean ± SD) of the SOD (A), MDA (B), GPx (C), and CAT (D) enzyme activities in the uterus tissues of different groups examined during three months. ***p < 0.001, and **P < 0.01 indicate a significant difference compared to the control group.

ER and PR Immunohistochemical assays

An immunohistochemistry assay was used to evaluate the uterus ER and PR expression (Fig. 3). Severe ER expression was observed in the nucleus of the endometrial epithelium (arrow tip) and glands (arrow) of uterine tissue of control group (Fig. 3; A1), while medium PR expression was found in the nucleus of cells below the endometrium (arrow tip) and glands (arrow) in this group (Fig. 3; A2). In addition, medium expression of ER and PR was found in the endometrial epithelium nucleus (arrow tip) and mucosal cells (arrow) of uterine tissue in T_1 (Fig. 3; B1 and B2). Moreover, medium ER expression was observed in the nucleus of mucosal cells of uterine tissue (arrow; Fig. 3; C1). It was also true for PR in the nucleus of mucosal cells (arrow) and glands (arrow tip) in T_2 (Fig. 3; C2).

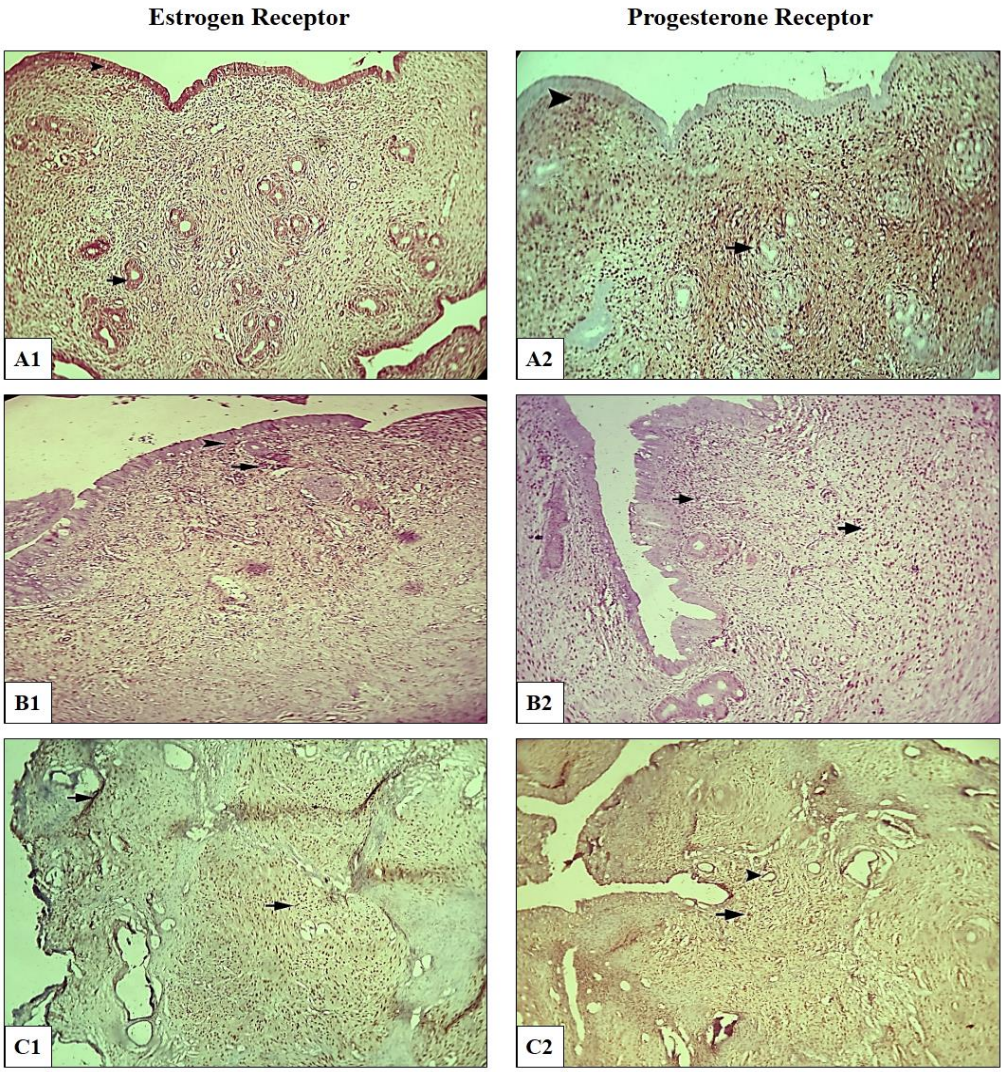


Fig. 3. The ER and PR immunohistochemistry assays of uterus tissue in the control and treatments. The ER expression (**A1**), and PR expression (**A2**) in uterine tissue of control. The ER expression (**B1**), and PR expression (**B2**) in uterus tissue of Treatment 1 (T_1). The ER expression (**C1**), and PR expression (**C2**) in uterus tissue of Treatment 2 (T_2; 40X magnification).

DISCUSSION

Due to the lifestyle of human beings, nowadays, air pollution in the form of $PM_{2.5}$ is known as the biggest threat to environmental health (Forouzanfar *et al*. 2016). $PM_{2.5}$ in addition to affecting the biological pathways and effects of the respiratory organs, has dangerous effects during pregnancy and the female reproductive system, since these particles can enter the bloodstream through the respiratory tract (Brown *et al*. 2002). It is important to know the exact and effective mechanism of pollutant particles on the tissue structure of the reproductive system to find effective prevention or treatment of injuries and also to measure alterations in ER and PR as an indicator in determining cancers including uterine cancer. Therefore, in this study, the effects of $PM_{2.5}$ on oxidative stress enzymes and the expression of uterine ER and PR in female Wistar rats were investigated. Specific PM-binding toxic compounds, such as PAHs, have been identified as carcinogenic and genotoxic, and have also been shown to impair endocrine functions such as the female reproductive system (Boström *et al*. 2002). Studies have shown that $PM_{2.5}$ and PM_{10}, which contains heavy metals and PAHs, may alter sex hormone levels of estrogen and can exhibit adverse effects on the human reproductive system factors. They are known as endocrine hormone destroyers that can affect the glands. Hypothalamus, testes, and ovaries are affected and interfere with the function of this system (Shang *et al.* 2019). In the present study, we also examined the concentration of $PM_{2.5}$, heavy metals, and PAHs. Our results showed high concentrations of these pollutants in the air. Loss of physiological balance between oxidants in antioxidants in the body following exposure to $PM_{2.5}$ air pollutants causes oxidative production reactions and cell damage. This phenomenon can cause a change in the structure of DNA, proteins,

and cell membranes. These factors also reduce the ability of arteries to dilate and increase blood pressure during pregnancy (Shang *et al*. 2019). The most vulnerable period against $PM_{2.5}$ is during pregnancy and embryo, during which embryonic and organ development occurs and the foetus lacks immune function during this period (Zheng *et al*. 2016). Exposure to 2.5 PM is generally directly associated with inhibition of phagocytosis, stimulation of the inflammatory response, and increased levels of oxidative stress (Terzano *et al*. 2010). The body's antioxidant defence system consists of three levels. The first level antioxidants prevent the formation of oxidative free radicals (ROS) and include the enzymes SOD, GPx, and proteins bound to the metals ferritin and ceruloplasmin. The second level that inhibit chain reactions and thus trap free radicals, include vitamin E, vitamin C, beta-carotene, uric acid, albumin, and bilirubin. The third level are enzymes that repair biomolecules such as DNA that have been damaged by free radicals (Esfahani *et al*. 2011). An imbalance between the production of free radicals and the status of antioxidants leads to oxidative damage to macromolecules, including fats and proteins. The increased level of ROS or reduced action of antioxidants or incomplete removal of ROS in cells lead to serious diseases in people. The activities of SOD, MDA, and GPx are useful indicators to reveal the level of oxidative stress that leads to dysfunction of normal cells (Banihashemrad *et al*. 2016). Numerous studies have shown that there is an association between $PM_{2.5}$, which contains high levels of metals or PAH, and elevated ROS production in cells (Manzo *et al*. 2011). Mitochondria are known as organs involved in maintaining redox balance (Blajszczak *et al*. 2017). The elevated ROS in the cell leads to inflammation and eventually apoptosis (Upadhyay *et al*. 2003). $PM_{2.5}$ exposure has been shown to specifically target mitochondria and induce mitochondrial ROS production in human cells (Rodríguez-Cotto *et al.* 2015). Studies on pairs exposed to higher levels of air pollution have shown methylation and the decrease in placental mitochondrial DNA (Janssen *et al.* 2015). Our study in line with findings of other authors, showed that the $PM_{2.5}$ in exposed mice led to oxidative damage in the body with a significant elevation in MDA, as well as a drop in SOD, GPx and CAT. Exposure to $PM_{2.5}$ during pregnancy leads to fetal weight loss, intrauterine growth restriction, and preterm delivery (Hjortebjerg *et al*. 2016; Trasande *et al*. 2016). There is a dependence between ERs and PRs in the cell that in the absence of estrogen, an upraise in the number of ERs and a decline in PRs can be expected or vice versa, and perhaps the amount of hormone in the tissue is affected by the number of receptors *(Pellicciari et al*. 2005). There is a significant relationship between the presence of the estrogen and progesterone receptors and some types of tumours which may help to early diagnosis and treatment (Pellicciari *et al.* 2005). Numerous studies have shown that $PM_{2.5}$ during maternal pregnancy leads to trophoblast invasion abnormalities and high vascular resistance through the impaired uterine coil artery repair, as well as the adaptive arterial angiogenesis and decreased uterine placental blood flow, which leads to these events that have severe impairment of foetal oxygenation and nutrition (van den Hooven *et al*. 2012). The most important molecular goal in clinical trials related to pregnancy and female reproductive function is to evaluate the functional level of ERs. ERs play an important role in uterine angiogenesis (Magness *et al*. 1996; Miller *et al*. 1999), and have important physiological effects on uterine vascular function especially during pregnancy (Lang *et al*. 2003). Even, $PM_{2.5}$ causes certain genetic or epigenetic abnormalities and leads to uterine fibroids (UF) (Lin *et al*. 2019). Our immunohistochemistry results also showed that Treatment 2 (T_2) had a significant decrease in ER, and PR of uterine tissue compared to the healthy control and Treatment 1 (T_1). $PM_{2.5}$ has the potential to induce tissue damage in the uterus. In-depth analysis suggests that oxidative stress, and ER-PR immunohistochemistry problems may play an important role in the effects of $PM_{2.5}$ on reproductive system damage, leading to adverse pregnancy outcomes.

CONCLUSION

Abnormal foetal growth, reduced birth weight, and rapid catch-up growth in childhood predispose to high morbidity in infants and subsequent risk of adult diseases. Therefore, it is important to know the factors involved in this process and prevent their risks on embryonic and postnatal development. Our evaluation of the $PM_{2.5}$ effect on the uterus of exposed female mice showed that $PM_{2.5}$ can lead to uterine damage by inducing oxidative damage and reducing the receptors involved in the function of estrogen and progesterone, and if pregnancy occurs there are dangerous risks to the foetus, so serious policies in today's societies should be established and implemented to quickly control the problem of air pollution.

DECLARATIONS

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Authors' contributions

N.Y.K., P.M. developed the idea and designed the experiments, N.Y.K., P.M., S.M.Z. conducted the experiments. A.E., P.M. analyzed the data. N.Y.K. wrote the manuscript. A.E, P.M., S.M.Z. helped through characterization of the samples. All authors confirmed the final manuscript before the submission**.**

Availability of data materials

All data analysed during this study are included in this published article.

Ethics approval and consent to participate

There are no "human subjects" in this study.

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