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Original Article

Adverse Impact of Ambient Particulate Matter on Cardiac Electrophysiology and the Lipid Profile in Rats

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ABSTRACT

Background: Particulate matter (PM) is an organic and inorganic mixture of particles of different sizes and chemical compositions. Positive correlations exist between the concentrations of air PM and respiratory and cardiovascular disorders, causing premature mortality and morbidity. This study was designed to evaluate the effects of PM on cardiac electrophysiology and the lipid profile in rats.

Methods: A total of 72 male Wistar rats (250–300 g) were divided into 6 groups: control (intratracheal instillation of 0.1-mL normal saline), PMA (intratracheal instillation of 0.5-mg/kg particles less than 10 μm [PM10]), PMB (intratracheal instillation of 2.5-mg/kg PM10), PMC (intratracheal instillation of 5-mg/kg PM10) twice at 48-hour intervals, calcium chloride (CaCl2) (140 mg/kg, intravenous), and isoproterenol (100 mg/kg, subcutaneous). After 48 hours, lead II electrocardiography was recorded and the inotropic and chronotropic properties of the heart and the incidence of arrhythmias were evaluated. Cardiac lipid parameters, including plasma cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides, and cardiac markers of myocardial infarction, creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) were measured.

Results: After the administration of PM10, there was a significant decrease in the voltage of the QRS complex and the R-R interval in comparison with the control group. There was a significant increase in the number of arrhythmias (premature ventricular contractions, ventricular tachycardia, and ventricular fibrillation) after PM10 administration. The administration of PM10 led to an increase in LDL, cholesterol, triglycerides, LDH, and CK-MB and a decrease in HDL in all the concentration groups.

Conclusions: PM10 can be introduced as an arrhythmogenic agent with the potential to affect the cardiac lipid profile by inducing cardiac damage and infarction. ***(Iranian Heart Journal 2021; 22(1): 33-41)***

KEYWORDS: Particulate matter, Arrhythmia, Inotropic, Chronotropic, Rat

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Recently, many epidemiologic studies have reported a positive correlation between the concentrations of particulate matter (PM) and premature death attributable to cardiovascular disease causes,[1-2] even with short-term PM exposure.[3] The morbidity and mortality caused by air pollution were first raised in the Meuse Valley air pollution episode in 1930, in which many people died within 3 days and after several investigations, it was concluded that exposure to PM could cause cardiovascular morbidity and mortality.[5] It is estimated that every 10 mg/m^3 increase in PM causes an increase in the risk of cardiovascular morbidity and mortality by 24% and 76%, respectively.[6] Long-term exposure to air pollution increases the rate of cardiopulmonary disorders, resulting in an estimated 800 000 early deaths annually worldwide.[7] Although the risk of respiratory dysfunction induced by PM is relatively greater than that caused by cardiovascular disorders, the absolute number of deaths attributable to PM is much higher for cardiovascular disorders than respiratory dysfunction.[8] Research has documented the adverse effects of short-term exposure to PM10 (particles < 10 μm) on pulmonary health; nonetheless, for early mortality, long-term exposure to PM2.5 (particles in the 2.5–10 μm range) is a stronger risk factor than PM10.[9]

The pathogenic mechanism responsible for the induction of adverse cardiovascular health effects is still unknown. Recent research has demonstrated that the direct entrance of PM into the systemic circulation could cause cardiovascular dysfunction. PM can cross the alveolar epithelial barrier into the systemic circulation and affect cardiovascular function.[10] Another suggested mechanism is that the activation of calcium/calmodulin-dependent protein kinase II by PM is associated with prolonged repolarization and cardiac arrhythmias.[11] Several studies have demonstrated that some arrhythmogenic agents can be used to induce arrhythmia models. For example, a high dose of calcium chloride (CaCl2) initiates arrhythmias directly and indirectly by modulating calcium channels and activating the sympathetic nervous system, respectively.[12] With regard to the increase in the incidence of health disorders, especially cardiovascular diseases, in provinces exposed to air pollution caused by dust particles, it is necessary to carry out more research into the mechanisms of the development of disorders caused by PM. Therefore, this study was designed to evaluate the effects of ambient PM on cardiac electrophysiology properties in rats.

METHODS

Animals

A total of 72 healthy male Wistar rats (250–300 g) were purchased from the Animal House of Ahvaz Jundishapur University of Medical Sciences. They were kept at 22 ± 1 °C, with 40–60% humidity and the 12/12 hour light-dark cycle. Food and water were available *ad libitum*, and all the experiments were approved by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. IR.AJUMS.REC.1394.612). The rats were randomly divided into 6 groups (n=8): a control group, which received an intratracheal instillation of 0.1-mL normal saline twice at 48-hour intervals; 3 PM10 exposure groups, consisting of a PM_A group, which received 0.5 mg/kg of PM10, a PM_B group, which received 2.5 mg/kg of PM10, and a PM_C group, which received 5 mg/kg of PM10 intratracheally twice at 48-hour intervals; and 2 positive control groups, which received well-known arrhythmogenic agents: a $CaCl_2$ group,

which received 140 mg/kg of $CaCl_2$ intraveneously,[14] and an isoproterenol group, which received 100 mg/kg of isoproterenol subcutaneously.[15]

PM Sampling

Ahvaz (capital of Khuzestan Province) is located in southwestern Iran in the vicinity of Saudi Arabia, Iraq, and Kuwait, which are the major sources of dust events in the Middle East.[24, 25] PM10 samples were collected using an air-pollution measurement device (Ecotec, Australia) located at least 5 m above the ground. Collected particles were recovered from the filters using surgical blades and then weighed. Finally, suspensions of the particles were prepared just before the intratracheal instillation, vortexed for 20 minutes, and then immediately instilled.

Exposure to PM10

The intraperitoneal anesthesia of the rats was done with the injection of a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg). After intratracheal intubation, mechanical ventilation (room air, 60 cycles/min, and 1 mL per 100 g of body weight tidal volume-rodent ventilator) was done. PM10 in all the concentrations was dissolved in 0.1-mL normal saline and administrated via intratracheal intubation. Then, the animals were maintained in an upright position for 2 minutes and then ventilated for 5 minutes to allow the fluid to drain into the respiratory tree. The same amount of normal saline was administered in the sham group. Each exposure protocol was applied twice at 4-hour intervals.[16]

Arrhythmia Model

The animals were anesthetized with a combination of ketamine (50 mg/kg) and xylazine (5 mg/kg) via the intraperitoneal route. After the prepping and draping of the rats with Betadine, the femoral vein was exposed and $CaCl_2$ (140 mg/kg) was injected for the induction of arrhythmia. In the isoproterenol group, arrhythmia was induced with the subcutaneous injection of isoproterenol (100 mg/kg) once daily for 2 days.

Method of Arrhythmia and Electrocardiographic (ECG) Parameter Recording

The standard bipolar limb lead II was recorded for the ECG analysis in a 15-minute period. Lead II ECG was recorded using Bio-Amp and monitored with a Power Lab system (AD-Instruments, Australia). The voltages of the QRS complex and the R-R interval were considered to represent the inotropic and chronotropic properties of the heart. Also, the number of ventricular premature beats, ventricular tachycardia, and ventricular fibrillation was calculated in a 15-minute period.

Histopathological Examination of the Heart

After 48 hours, heart tissue was removed from the rats of all the concentrations of PM10 groups, fixed with formaldehyde, and then embedded into paraffin. Tissue sections at 5 µm were stained with hematoxylin and eosin (H&E) for the microscopic (OLYMPUS Microscope) examination of morphological changes.

Measurement of Cardiac Lipid Parameters

At the end of the experiment, blood samples were collected through the cardiac puncture of the anesthetized rats. Thereafter, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels were assayed according to the instruction of their specific kits (ZellBio, Germany).[17]

Measurement of the Activity of Cardiac Enzymes

The activity levels of CK-MB and LDH were detected using the creatine kinase MB isoenzyme assay kit and lactate dehydrogenase assay kit, respectively,[18] using an automatic biochemical analyzer in accordance with the manufacturer's protocols.

Statistical Analysis

Data comparisons were performed using the ANOVA test and expressed as mean±SEM. A *P value* of less than 0.05 was considered significant statistically.

RESULTS

Effect of PM10 on the Chronotropic and Inotropic Properties of the Heart

After 2 administrations of PM10, there was a significant decrease in the inotropic index (voltage of the QRS complex) only in the PM_C (5 mg/kg PM10) group in comparison with the control group. There was no significant effect by the other concentrations of PM10 on the voltage of the QRS complex (Fig. 1). Additionally, the chronotropic index (the R-R interval) significantly increased in all 3 concentration groups compared with the control rats (Fig. 2).

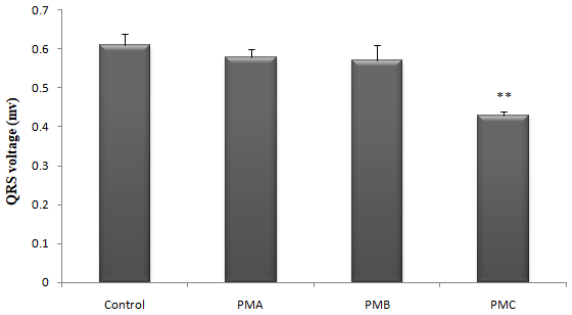

Figure 1: Effect of PM10 on the voltage of the QRS complex is shown herein.
Control (saline, 0.1 mL), PMA (0.5 mg/kg PM10), PMB (2.5 mg/kg PM10), and PMC (5 mg/kg PM10) Values are presented as mean±SEM (n=8).
*P < 0.05 and **P < 0.01 vs Control

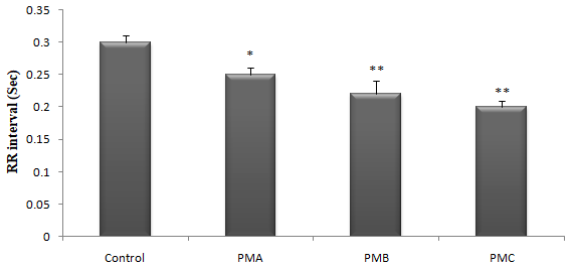

Figure 2: Effect of PM10 on the R-R interval is illustrated herein.
Control (saline, 0.1 mL), PMA (0.5 mg/kg PM10), PMB (2.5 mg/kg PM10), and PMC (5 mg/kg PM10) Values are presented as mean±SEM (n=8).
*P < 0.05 and **P < 0.01 vs Control

Effect of PM10 on the Incidence of Arrhythmia in Comparison With $CaCl_2$ and Isoproterenol

$CaCl_2$ and isoproterenol are well-known arrhythmogenic agents for the induction of an arrhythmia model. In this study, the administration of 140-mg/kg $CaCl_2$ and 100 mg/kg isoproterenol showed all 3 types of arrhythmias (ventricular premature beats, ventricular tachycardia, and ventricular fibrillation) in various numbers of incidences. Surprisingly, the administration of PM10 led to a significant increase in the incidence of arrhythmia in comparison with the control. Nevertheless, these values were significantly lower in comparison with the $CaCl_2$ and isoproterenol groups (Fig. 3).

Effect of PM10 on Cardiac Histopathology

The heart tissues harvested from the control group demonstrated that there was no myocardial damage and cardiac muscle fibers were regular. In contrast, the PM10 exposures in different concentrations resulted in irregularly arranged fibers with the infiltration of inflammatory cells. The myocardial injuries (Fig. 4), confirmed the adverse effects of PM10 on the cardiovascular system.

Effect of PM10 on the Serum Lipid Profile

The PM10 rats exhibited a significant increase in the levels of serum LDL, cholesterol, and triglycerides and a significant decrease in the

HDL level in all the concentration groups, indicating an increase in cardiac risk compared with the control groups (Fig. 4).

Effect of PM10 on CK-MB and LDH

Cardiac enzyme activity was determined using CK-MB and LDH assay kits to evaluate myocardial damage. The results demonstrated that PM10 administration in all the concentrations resulted in a significant increase in CK-MB and LDH activities compared with the control group (Fig. 5).

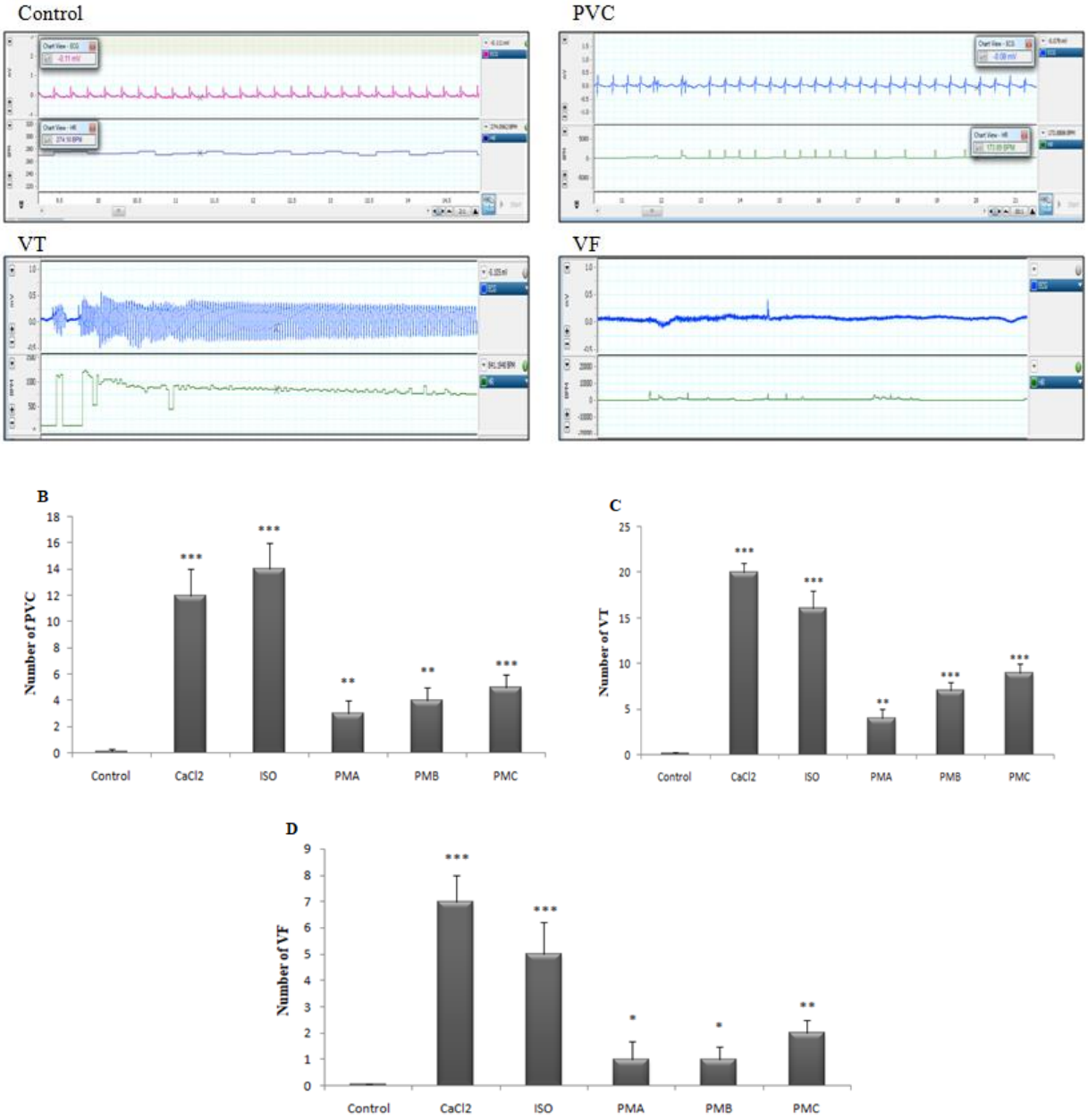

Figure 3: Sample tracing electrocardiographic data from the rats are presented. (A): Effects of PM10 on the frequency of (B) PVC, (C) VT, and (D) VF

Control (saline, 0.1 mL), $CaCl_2$ (140 mg/kg), isoproterenol (100 mg/kg), PMA (0.5 mg/kg PM10), PMB (2.5 mg/kg PM), and PMC (5 mg/kg PM10)

*P < 0.05, **P < 0.01, and ***P < 0.001 vs Control

PVC, Ventricular premature beats; VT, Ventricular tachycardia; VF, Ventricular fibrillation

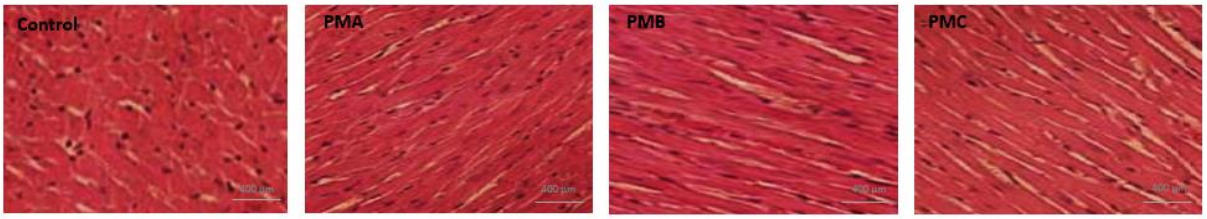

Figure 4: Representative hematoxylin and eosin (H&E) staining of heart tissue is illustrated in the control (saline, 0.1 mL), PMA (0.5 mg/kg PM10), PMB (2.5 mg/kg PM), and PMC (5 mg/kg PM10) rats.

Scale = 400 µm

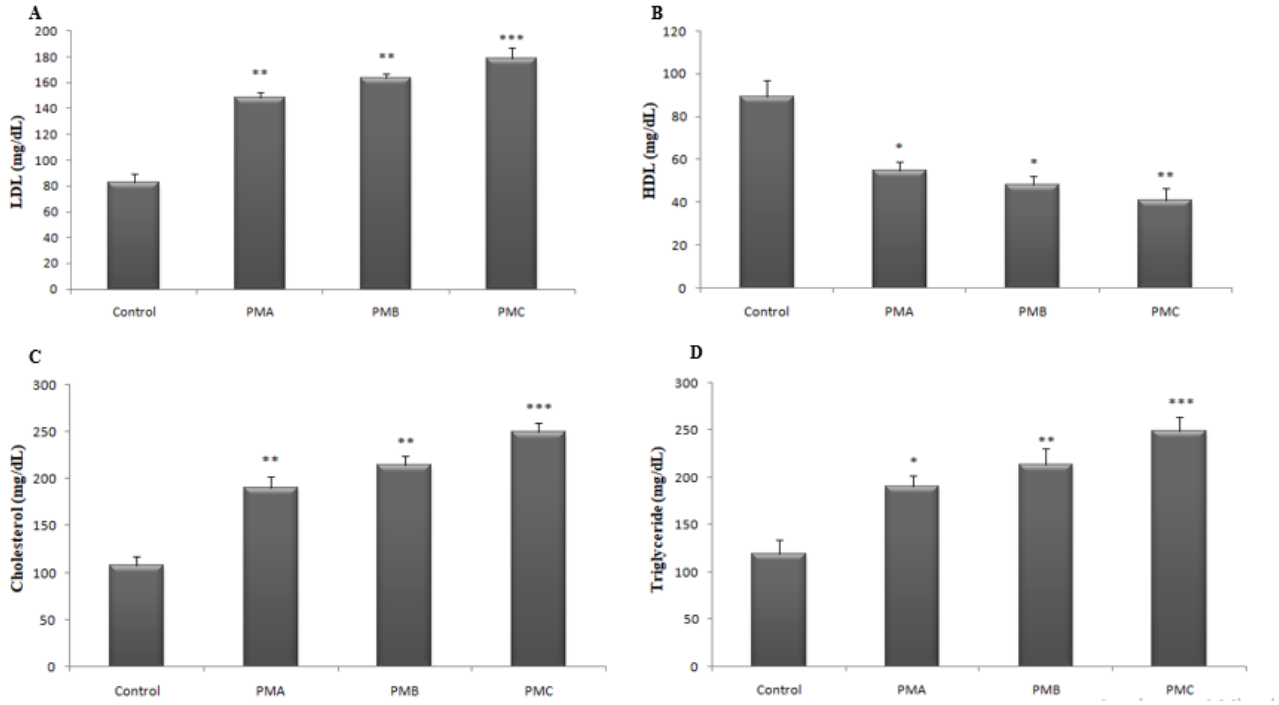

Figure 5: Effects of PM10 on (A) LDL, (B) HDL, (C) cholesterol, and (D) triglycerides are illustrated herein. Control (saline, 0.1 mL), $CaCl_2$ (140 mg/kg), isoproterenol (100 mg/kg), PMA (0.5 mg/kg PM10), PM_B (2.5 mg/kg PM), and PM_C (5 mg/kg PM10).
Values are presented as mean±SEM (n=8).
*P < 0.05, **P < 0.01, and ***P < 0.001 vs Control
LDL, Low-density lipoprotein; HDL, High-density lipoprotein

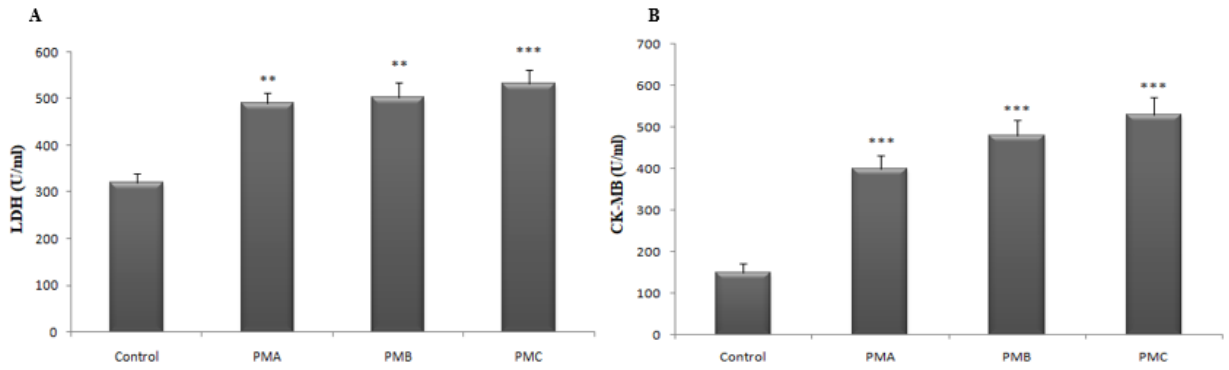

Figure 6: Effects of PM10 on (A) LDH and (B) CK-MB are illustrated herein.
Control (saline, 0.1 mL), $CaCl_2$ (140 mg/kg), isoproterenol (100 mg/kg), PMA (0.5 mg/kg PM10), PM_B (2.5 mg/kg PM), and PM_C (5 mg/kg PM10).
Values are presented as mean±SEM (n=8).
*P < 0.05, **P < 0.01, and ***P < 0.001 vs Control
LDH, Lactate dehydrogenase; CK-MB, Creatine kinase-MB; $CaCl_2$, Calcium chloride

DISCUSSION

The results of the current study documented that PM could be introduced as an arrhythmogenic agent with the potential to affect the cardiac lipid profile for the induction of cardiac damage and infarction.

PM pollution, as a major risk for health disorders, remains a public health concern worldwide.[19] The cardiopulmonary system is the main target for the adverse effects of PM both in chronic and acute exposures.[20] Dianat et al[21] (2016) showed that PM10 exposure caused a significant increase in the P-R interval, QTc, and blood pressure in rats. Radmanesh et al[22] showed that PM exposure increased the number and duration of arrhythmias in a cardiac ischemia/reperfusion model. We designed the current study to evaluate the possible arrhythmogenic properties of PM10 in 2 acute exposures in 48 hours. We also used $CaCl_2$ and isoproterenol, as well-known arrhythmogenic agents, as positive control groups and compared the incidence of arrhythmia between the PM10 exposure group and $CaCl_2$- and isoproterenol-administrated rats. Our results showed that the incidence of ventricular premature beats, ventricular tachycardia, and ventricular fibrillation increased significantly in the groups that received $CaCl_2$ and isoproterenol. Surprisingly, the administration of PM10 also induced arrhythmia in all the concentrations, with the highest incidence in the 5-mg/kg dose; still, the arrhythmia incidence was significantly lower than that in the $CaCl_2$ and isoproterenol groups. In the present investigation, electrophysiology parameters, including the voltage of the QRS complex and the R-R interval, were evaluated to assess the inotropic and chronotropic properties of the heart using ECG recording. PM10 exposure caused a significant decrease in the voltage of the QRS complex only in the group that received 5 mg/kg of PM10. Nonetheless, the chronotropic effects in all the concentration groups significantly increased compared with the control rats, which suggested that PM exerted negative effects on cardiac contractility and heart rate. A decrease in inotropic properties led to a decrease in stroke volume and left ventricular end-diastolic pressure, which along with tachycardia, may lead to arrhythmia. In line with our study, Radan et al[23] (2019) showed that the heavy metal in PM10 could disrupt the electrical and mechanical activities of the heart. Heavy metals in PM have a wide range of toxicity, which could lead to oxidative stress, enzymatic inhibition, and lipid peroxidation

and finally result in cardiomyopathy and cardiovascular disorders. Our study also demonstrated that PM10 exposure had an adverse effect on the lipid profile, including LDL, HDL, cholesterol, and triglycerides, which was associated with an increase in LDH and CK-MB enzyme activity and, thus, suggested myocardial damage. We also found that PM10 administration led to increased lipid levels in the serum and cardiac tissues. Hypercholesterolemia can increase the overproduction of free radicals, augment mitochondrial respiration, and lower the antioxidant status, which could lead to cardiac damage as was shown by the elevation in the CK-MB level in our PM10 exposure group.[24] In support of this hypothesis, several studies have documented that the inhalation of PM produces a wide range of deleterious effects by increasing reactive oxygen species, which can cause the lipid peroxidation of cell membranes and the depletion of protein sulfhydryls. PM exposure can affect calcium and sulfhydryl homeostasis and trigger a deleterious cycle of oxidative stress and inflammation in the target tissues.

CONCLUSIONS

In summary, the current study demonstrated that PM10 could be introduced as an arrhythmogenic agent with the potential to increase lipid levels and induce cardiac damage and infarction.

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Conflict of Interest: None declared.

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