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Impact of particulate matter 2.5 on the liver function of mice

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Abstract. – OBJECTIVE: The aim of this study was to evaluate the impact of particulate matter 2.5 (PM2.5) on liver function at the animal level and to study its impact targets.

MATERIALS AND METHODS: 60 male and female BALB/c mice of SPF grade, aged 6-8 weeks, were randomly divided into four groups, with 15 mice in each, including the normal saline control group, the PM2.5 low dose group [2 μg/(100 g/d)], the PM2.5 medium dose group [8 μg/(100 g/d)] and the PM2.5 high dose group [16 μg/(100 g/d)]. Each day, 0.9% saline or PM2.5 particles were administered through the nasal route, and samples were taken after 3 weeks of continuous exposure. Hematoxylin-eosin staining (HE) was used to observe the liver damage caused by PM2.5. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were detected by using an automatic biochemical analyzer to detect the content of liver glycogen and blood glucose. Multiple indicators were observed, including plasma tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) levels, oxidative stress response indicators reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD) detection, RT-PCR and Western blot detection of glycogen synthase (GS), glucokinase (GK), nuclear factor erythroid 2-related factor 2 (*Nrf2*) expression and phosphorylation level of phospho-c-Jun N-terminal kinases (p-JNK).

RESULTS: PM2.5 can cause damage to the liver by increasing PM2.5 concentrations, raising the metabolic rate of liver cells, resulting in a substantial amount of inflammatory infiltration and vacuolar degeneration of cells, and increasing the liver/body weight. TNF-α and IL-6 inflammatory factor expression increased ($p<0.05$). An increase in the serum ALT and AST levels were also observed. The blood glucose of mice increased, whereas the content of liver glycogen declined ($p<0.05$). ROS, MDA, and SOD levels all increased considerably. PM2.5 can drastically lower the expression of GS and GK, increase the expression of *Nrf2*, and raise the phosphorylation level of p-JNK ($p<0.05$).

CONCLUSIONS: PM2.5 can induce oxidative stress in mouse liver through the *Nrf2*/JNK pathway, induce liver inflammation in mice, and inhibit glycogen synthesis.

Key Words:
PM2.5, Oxidative stress, *NRF2*/JNK pathway, Inflammatory response.

Introduction

Epidemiological studies[1-3] have confirmed that the onset and fatality of various diseases are closely related to air pollution. Particulate matter 2.5 (PM2.5) refers to particulate matter with an aerodynamic diameter of not more than 2.5 μm. It is one of the major air pollutants, posing a severe threat to human health[4]. The composition of PM2.5 is relatively complex, including organic carbon, elemental carbon, inorganic ions, heavy metals, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and biological components[5]. After inhalation, PM2.5 can be transferred from the lungs to the systemic blood circulation system, ultimately damaging the extrapulmonary organs[6]. The impact of PM2.5 on liver illnesses has secured public attention. The liver, a key detoxifying organ in the human body, plays a key role in metabolism. It is also able to store glycogen and synthesize released proteins. After entering the body, PM2.5 travels to the liver *via* blood circulation, resulting in liver damage of various degrees[7]. Humans' or animals' exposure to PM2.5 in the environment can cause inflammatory damage and extensive

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oxidative damage to various organs and tissues including the liver[8]. According to research, once PM2.5 particles enter the body through the lungs, they will adhere to lung tissues and interact with lung epithelial cells as well as lung macrophages, generating and releasing a great amount of cytokines and reactive oxygen species (ROS)[9]. The generated ROS enters the body *via* the respiratory tract or other passages, and the free radicals carried by PM2.5 might reach the liver *via* blood circulation, interact with liver cells, and eventually induce inflammatory response in the body. A sequence of oxidative damages and inflammatory responses occur in the liver cells, resulting in lipid peroxidation in the cell membrane of the liver tissue, which impair the antioxidant capacity of the liver tissue or increase the oxidative stress response, thus exerting impacts on liver function[9,10]. A study[11] has shown that long-term exposure of experimental mice to PM2.5 can boost ROS production in the liver of the mice; PM2.5 exposure combined with high-fat diet (HFD) can increase the rate of liver fibrosis as well the degree of liver damage.

PM2.5 pollution can activate a variety of undesirable signaling pathways in the liver, lungs, adipose tissue, and blood vessels, which are associated with oxidative stress, endoplasmic reticulum stress, and inflammatory response[12,13]. There are a series of antioxidant defense mechanisms in the body, including such antioxidant enzymes as superoxide dismutase (SOD), catalase, glutathione (GSH), vitamins A, C, and E. When the defense mechanisms fail to resist ROS production, oxidative stress will be formed, inducing a series of cellular reactions, including proliferation, growth arrest and death. The level of active oxygen in the cell has a direct bearing on the survival of the cell. Low concentration of active oxygen promotes cell growth, while high concentration of active oxygen leads to cell death, including apoptosis and necrosis.

The nuclear factor erythroid 2-related factor 2 (*Nrf2*), coded by *Nfe2l2*, is a transcription factor of cap-n-collar (*CNC*) family, which share a common conserved region, collectively called the basic leucine zipper (*bZIP*) protein. The *bZIP* protein regulates the expression of antioxidant proteins, thereby preventing inflammation and oxidative damage[14]. It has been believed that early and transient activation of phospho-c-Jun N-terminal kinases (*p-JNK*) can play multiple roles in anti-apoptosis and pro-differentiation[15]. Only the continuous activation of *JNK* can initiate the occurrence of apoptosis. Therefore, the conclusion may be that under mild stress, the level of active oxygen produced at the time is so low that it cannot continue to activate *JNK*. Instead, it activates some signaling molecules that promote survival, including nuclear factor kappa B (*NF-κB*) and phosphoinositide 3-kinase (*PI3K*). *JNK* has a protective impact on cells; when the level of active oxygen rises, *JNK* continues to activate, causing apoptosis and cell necrosis. It has been proven in studies[16,17] that exogenous oxidants or failure of self-defense systems will lead to excessive ROS production, which can generate oxygen free radicals and cause oxidative stress responses. Oxygen free radicals and oxidative stress responses are associated with the causative factors of various diseases, such as liver injury, heart disease, immune injury, inflammation, cancer, etc[18,19]. The degree of oxidative stress can be reflected by malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD)[20].

There is no systematic report on the mechanism of liver damage caused by PM2.5. Therefore, this study explored the potential mechanism of PM2.5-induced liver inflammation and whether oxidative stress-mediated liver damage is regulated by the *Nrf2/JNK* pathway, thereby providing insights for the prevention and treatment of liver inflammation-related diseases caused by PM2.5.

Materials and Methods

Experimental Materials

PM2.5 particles were purchased from Boulder Corporation (La Jolla, CA, USA) suspended in phosphate buffer saline (PBS) for 24 hours, and ultrasonicated with nearly 40 W for 20 minutes, exposed to centrifugation at 13,000×g and 4°C for 10 minutes, and filtered with a 0.22 um syringe filter[21].

Animals

60 BALB/c mice of SPF grade, half male and half female, aged 6-8 weeks, weighing 20-30 grams, were kept in the Animal Laboratory of the Tenth People's Hospital, at a temperature of 20-24°C and a humidity of 40%-60%. After having adapted to feeding for one week, the mice were randomly divided into 4 groups, including the normal saline control group, the PM2.5 low dose group (PM2.5-L) [2 μg/(100 g/d)], the PM2.5 medium dose group PM2.5-M) [8 μg/(100 g/d)] and

the PM2.5 high-dose group (PM2.5-H) [16 μg/(100 g/d)]. Each day, 0.9% saline or PM2.5 particles were administered through the nasal route for 3 weeks of continuous exposure[22].

Hematoxylin-Eosin (HE) Staining and TUNEL

After the blood was taken, the mice were killed by neck-breaking, then the liver was carefully separated. A piece of liver tissue was transected at 0.5 cm from the right lobe of the liver and fixed with 10% neutral formalin. The tissue was dehydrated and embedded in paraffin. The tissue was cut into 5 um serial slices, which were baked in a 60°C incubator for 1 hour, dewaxed and hydrated, HE stained and sealed. TUNEL testing is performed according to the kit instructions. Observe and take pictures under an optical microscope[22]. TUNEL tests were performed according to the kit instructions. Observation was made and pictures were taken under an optical microscope.

Liver Function Test

After anesthesia, 4 ml of blood was collected from the inferior hepatic vena cava, and the alternative lengthening of telomeres (ALT) and aspartate aminotransferase (AST) levels were detected using an automatic biochemical analyzer. The kit was used to detect the content of liver glycogen in mice.

ELISA

Blood samples were collected from the inferior hepatic vena cava. Plasma was separated *via* centrifugation and stored at -20°C until use. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentrations of plasma TNF-α and IL-6. The operation steps were completed according to the kit instructions.

Western Blot

Western blot analysis was performed to determine the levels of glycogen synthase (GS, ab40810), glucokinase (GK, ab88056), Nrf2 (ab89443), JNK (ab199380), p-JNK (ab47337) (all from Abcam, UK). The liver tissue pieces were thoroughly triturated with liquid nitrogen, and a protein extraction mixture was added. After homogenization, the mixture was sonicated under ice bath at 4°C, centrifugated at 13,000 r/min for 10 min, and then the supernatant was taken. The bovine clostridial abomasitis (BCA) protein assay kit was used to detect the protein concentration of each sample. SDS-polyacrylamide gel electrophoresis was run at a low constant voltage of 80 mV for 30 min and then a high constant voltage of 120 mV until bromophenol blue reached the edge of the gel. The membrane was cut and transferred to a constant current of 300 mA for 90 minutes. After the membrane was transferred, it was washed with methanol, double-distilled water and Tris buffered saline Tween (TBST) in sequence, and then blocked with 5% BSA for 1 hour. The primary antibody was added at 4°C overnight, and the mouse anti-ratβ-actin monoclonal antibody was used as internal reference. When the membrane was washed with TBST, and goat anti-rabbit secondary antibody was added and the goat anti-mouse secondary antibody was taken as the internal reference. Then the gel was exposed at room temperature for 1 hour. After the chemiluminescent reagent was added dropwise on the polyvinylidene fluoride (PVDF) membrane for imaging, the expression of the target protein was indicated by the ratio of the absorbance value of the target protein band to the corresponding β-actin band.

Real Time PCR

Samples were added to RNAiso Plus (Takara Bio Inc., Beijing, China) for RNA extraction. RNA samples were reversely transcribed using the Invitrogen reverse transcription kit (Waltham, MA, USA). RT-qPCR was performed using SYBR Green. The determination was performed in a total volume of 20 μl, which contained 0.8 μl of each primer. PCR conditions were 95°C for 5 minutes, 95°C for 10 s, 58°C for 20 s and 40 cycles, starting from 72°C to 0.5°C for 5 cycles each time and 80 cycles in total.

Tests Related to Oxidative Stress

MDA was detected by the thiobarbituric acid method. The steps were completed according to the kit instructions. GSH can produce a yellow compound in reaction with dithiodinitrobenzoic acid and mercapto compounds for quantitative colorimetric determination. The steps were completed according to the kit instructions. SOD activity was detected using xanthine oxidase method, with the steps completed according to the kit instructions. For ROS determination, 10 mg of nitro blue tetrazolium (NBT) was dissolved in 100 ml of PBS (pH=7.2), stirred at room temperature for 1 h, and then filtered with a 0.2 mm filter membrane, which was 0.1% NBT solution. The sample was tested with an equal volume of 0.1% NBT solution added. It was maintained in 37°C water bath for 1 hour, and then with 50% of

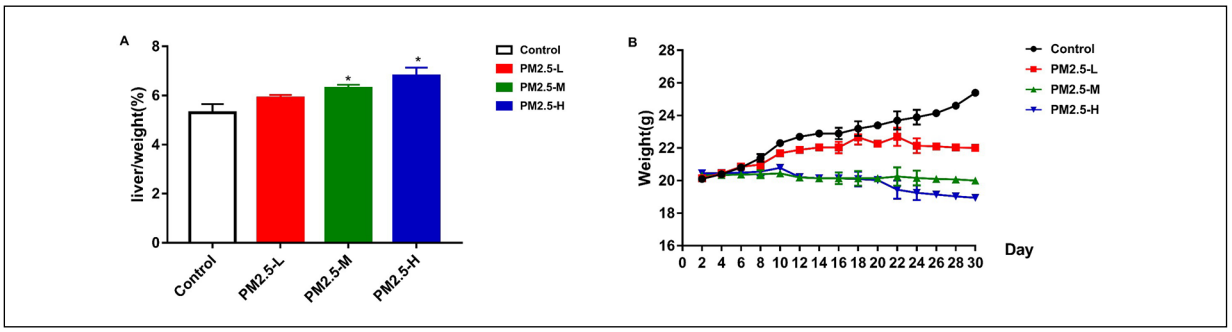

Figure 1. Effect of PM2.5 on mouse body weight, liver/body weight. **A,** The effect of PM2.5 on the body weight of mice. **B,** Effects of PM2.5 on liver/body weight of mice. Data were expressed as mean ± SEM (n = 12), *$p < 0.05$ *vs.* control, **$p < 0.01$ *vs.* control.

the acetic acid of the sample volume added twice, subjected to the 450 nm colorimetric assay.

Statistical Analysis

Normally distributed measurement data was represented by mean plus or minus standard deviation (). Student's *t*-test was used to compare two groups. For more than two groups, a one-way ANOVA was used to evaluate statistical significance ($p<0.05$). GraphPad Prism Version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA) was used to perform statistical analysis.

Results

Effect of PM2.5 on Mouse Body Weight, Liver/Body Weight

We investigated the weight of mice and the organ coefficients of the liver to understand how PM2.5 affected them. The findings revealed that the mice managed to keep gaining weight. According to control references, the organ coefficients of mice in the PM2.5 and PM2.5-H groups increased. The most noticeable reduction was observed in the PM2.5-H group (Figure 1A-B), indicating that PM2.5 lowers the mice's liver weight and body weight.

Effect of PM2.5 on Mouse Liver Histomorphology

We stained the livers of the mice with HE staining to visually assess the effect of PM2.5 on liver damage (Figure 2A-D). The results demonstrated that PM2.5 can cause liver damage and tissue congestion (black arrow), inflammatory infiltration (blue arrow), and cellular vacuole degeneration (yellow arrow) in mice (Figure 2A-D), implying that PM2.5 causes liver injury in animals.

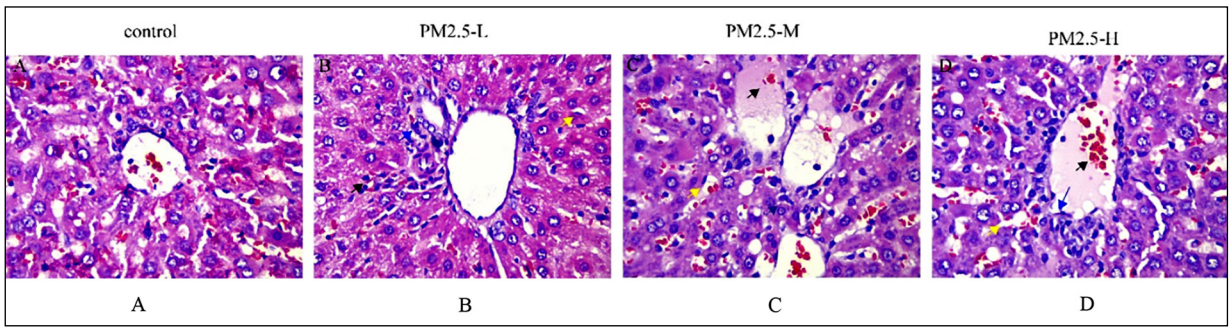

Figure 2. The effect of PM2.5 on mouse liver histomorphology. The sections of the liver were stained by HE. **A,** Control representative pictures (Magnification: 100×). **B,** Representative pictures of PM2.5-L (Magnification: 100×). **C,** Representative pictures of PM2.5-M (Magnification: 100×). **D,** Representative pictures of PM2.5-H (Magnification: 100×). The organ coefficients of mice have increased in PM2.5, and PM2.5-H group. We observed a slow rising, and then losing weight with time, the most obvious decline in the PM2.5-H group (Figure 1A and B), suggesting that PM2.5 affects liver weight and body weight of the mice.

The Effect of PM2.5 on Liver Ulcer in Mice

TUNEL labeling of mouse liver cells was utilized to determine the degree of PM2.5-induced liver damage (Figures 3A-E). There was no evident cellular overcoming in the control group (Figure 3A), but we did see a steadily increasing rate of TUNEL-positive cells in the PM2.5 treatment group from the model (Figure 3B-D), compared to the control group, indicating that PM2.5 triggers apoptosis of liver cells in mice.

Effect of PM2.5 on Liver Function of Mice Liver

The liver damage caused by PM2.5 will also weaken mouse liver function. As a result, we investigated the impact of PM2.5 on mouse liver function (Figure 4A-D). The results demonstrated that PM2.5 elevated the levels of ALT and AST in a concentration-dependent way when compared to the control group. The quantity of liver glycogens was dramatically reduced when compared to the control group, with the PM2.5-H group having the greatest drop. Meanwhile, we discovered that treating the animal with PM2.5 dramatically elevated blood glucose levels (Figure 4C). These findings imply that PM2.5 causes liver damage in mice.

Effect of PM2.5 on the Content of TNF-α and IL-6 in Mouse Liver

The role of PM2.5 in the control of inflammation was then investigated further. We measured the levels of TNF-α and IL-6 in the livers of mice and found that, when compared to the control group, the level of TNF-α in the PM2.5 group was much higher (Figure 5A-B), and the difference was statistically significant ($p<0.05$). We found that the higher the dosage of PM2.5, the more noticeable the difference from the control group (Figure 5A), and the difference was statistically significant ($p<0.05$). These findings imply that PM2.5 causes inflammation in mice.

Effect of PM2.5 on the Expression of Nrf2/NK Pathway-Related Proteins and mRNA in Mouse Liver

Then, we attempted to investigate the mechanism through which PM2.5 causes liver damage

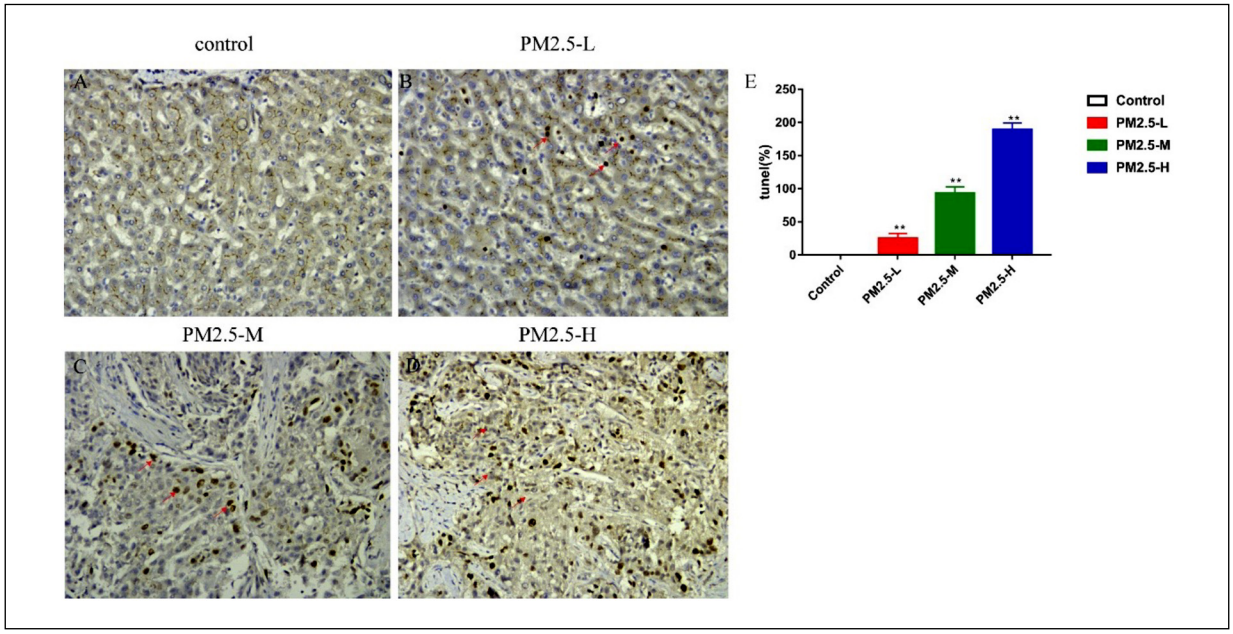

Figure 3. The effect of PM2.5 on mouse liver apoptosis. The sections of the liver were stained by Tunel. **A,** Control representative pictures (Magnification: 100×). **B,** Representative pictures of PM2.5-L (Magnification: 100×). **C,** Representative pictures of PM2.5-M (Magnification: 100×). **D,** Representative pictures of PM2.5-H (Magnification: 100×). **E,** Quantitative analysis of the number of apoptotic cells.Data were expressed as mean ± SEM (n = 12), *$p<0.05$ *vs.* control, **$p<0.01$ *vs.* control. There was no evident cell overcoming in the control group (Figure 3A) and we observed the gradually increased rate of TUNEL-positive cells in the PM2.5 treatment group from the model (Figure 3B-D), compared with the control group, suggesting that PM2.5 induces apoptosis of liver cells in the mice.

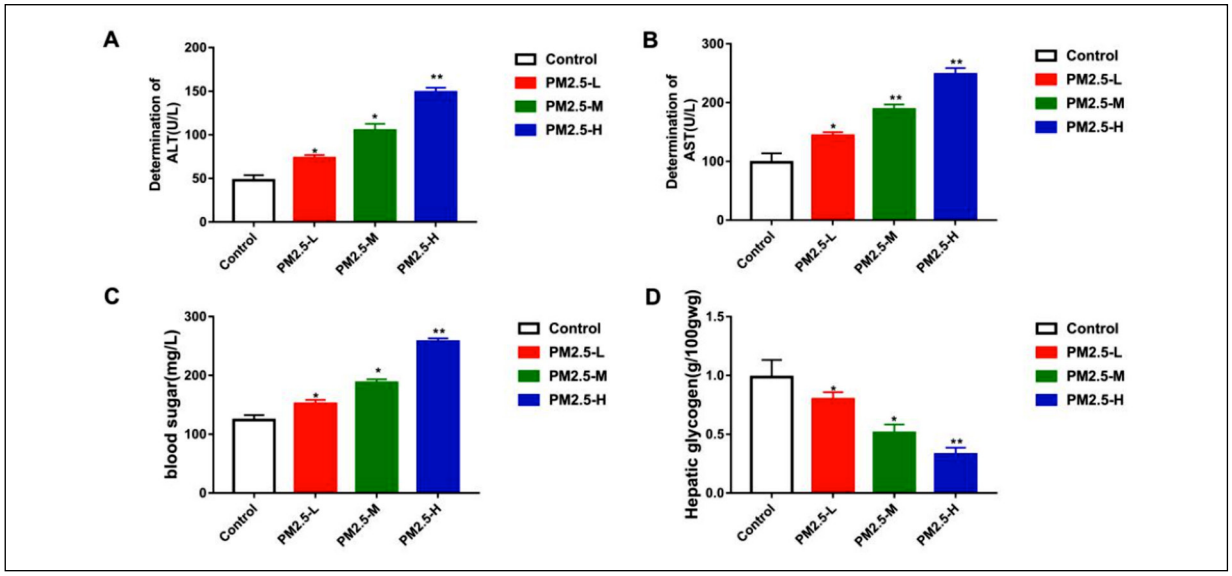

Figure 4. The effect of PM2.5 on liver function in mice liver. **A,** Determination of serum ALT content. **B,** Determination of serum AST content. C. Determination of liver glycogen content. D. Determination of blood sugar. Data were expressed as mean ± SEM (n = 12), **p*<0.05 *vs.* control, ***p*<0.01 *vs.* control. The results showed that the levels of ALT and AST were significantly increased by PM2.5 compared with the control group in a concentration-dependent manner. Compared with the control group, the number of liver glycogens was significantly reduced, and the PM2.5-H group had the largest decrease.

and inflammation. The levels of *Nrf2*, *JNK*, *p-JNK*, glucokinase (GK), glycogen synthase (GS) protein, and mRNA were determined using Western blot and RT-PCR. The findings are depicted in Figures 6A-E and Figure 7A-E. The protein and mRNA expression of *Nrf2* in the PM2.5 group were much higher than those in the control group (Figure 6D, 7C), and the difference was statistically significant ($p<0.05$). Compared with the control group, the phosphorylation level of *JNK* in PM2.5 groups increased significantly (Figure 6E), the difference was statistically significant ($p<0.05$). The mRNA level of *JNK* increased significantly (Figure 7B). Compared with the control group, PM2.5 group GS protein and mRNA expression were significantly reduced (Figures 6C, 7D), the difference was statistically significant ($p<0.05$). Compared with the control group, the expression of GK protein and mRNA in the PM2.5 group was significantly reduced (Figures 6B, 7A), the difference was statistically significant ($p<0.05$). These data indicate that PM2.5 regulates *Nrf2/JNK* signaling in the liver of the mice.

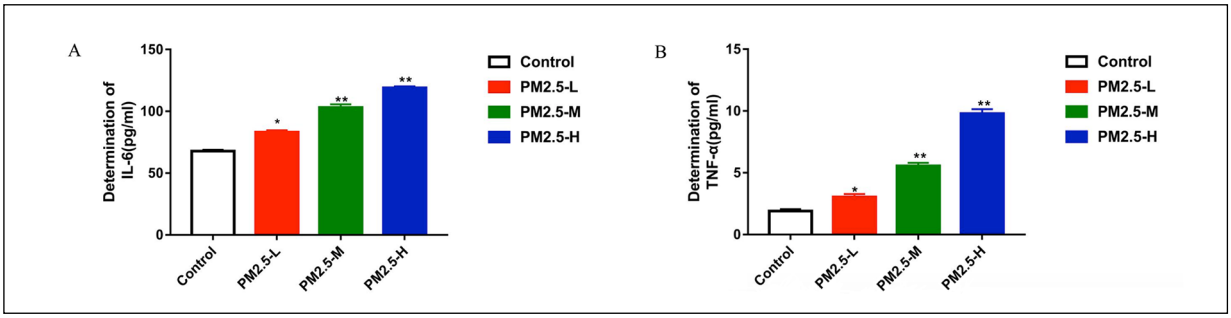

Figure 5. The effect of PM2.5 on the levels of TNF-α and IL-6 in mouse liver. **A,** Determination of IL-6 in mice. **B,** Determination of TNF-α in mice.Data were expressed as mean ± SEM (n = 12), **p*<0.05 *vs.* control, ***p*<0.01 *vs.* control.

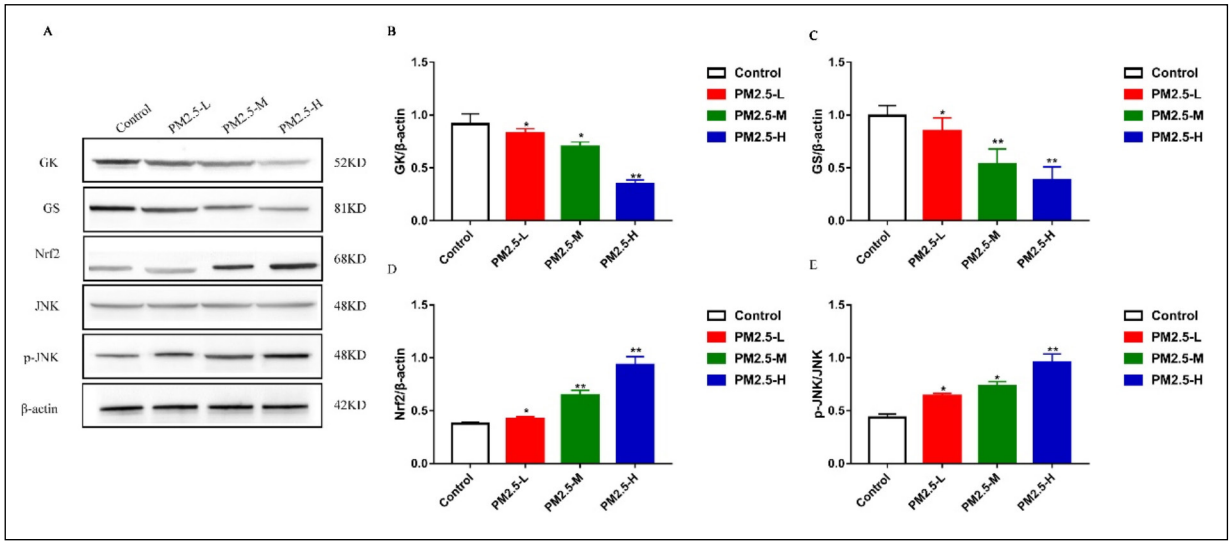

Figure 6. The effect of PM2.5 on the expression of Nrf2/JNK pathway-related proteins in mouse liver. The expressions of Nrf2, GK, GS, JNK and p-JNK protein were detected by Western blot analysis. **A,** The antibody-reactive bands of Nrf2, GK, GS, JNK and p-JNK. **B,** Quantitative analysis of GK levels. **C,** Quantitative analysis of GS levels. **D,** Quantitative analysis of Nrf2 levels. **E,** Quantitative analysis of p-JNK levels. Data were expressed as mean ± SEM (n=12), **p*<0.05 *vs.* control, ***p*<0.01 *vs.* control.

Effect of PM2.5 on Oxidative Stress Response in Mouse Liver

Furthermore, the levels of ROS, MDA, and SOD in mouse liver tissue were measured, and the findings revealed that (Figure 8), as compared to the control group, SOD expression in the PM2.5 group was much lower (Figure 8C), and the difference was statistically significant

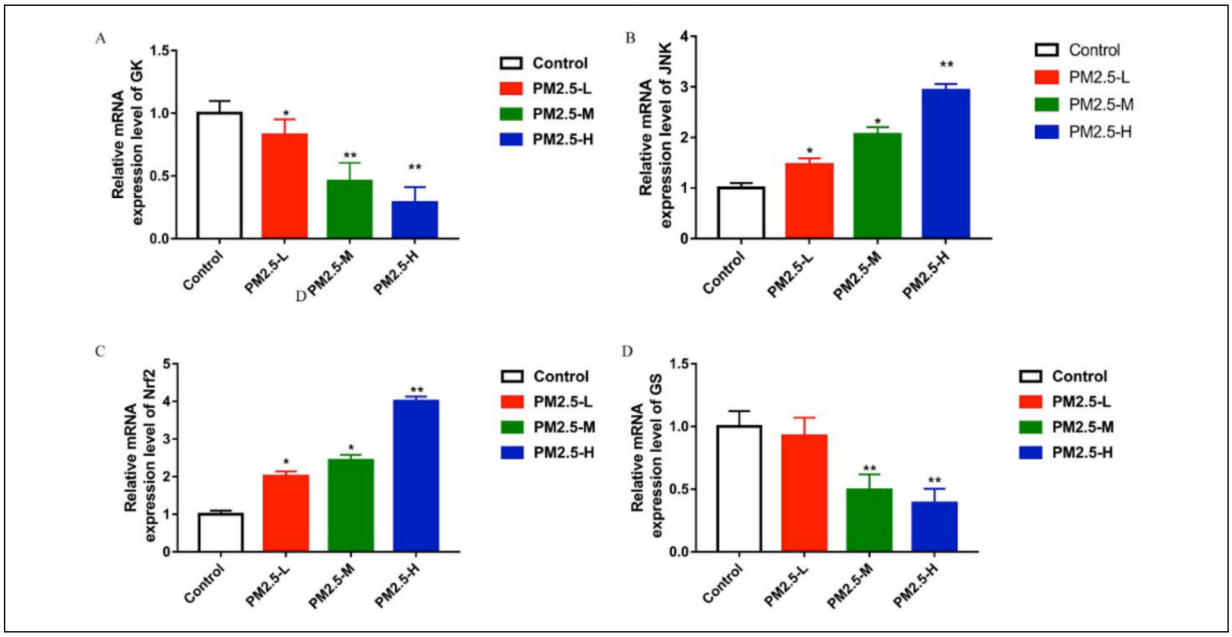

Figure 7. Effect of PM2.5 on mRNA expression of Nrf2/JNK pathway in mouse liver **A,** Quantitative analysis of GK mRNA levels. **B,** Quantitative analysis of Nrf2 mRNA levels. **C,** Quantitative analysis of JNK mRNA levels. **D,** Quantitative analysis of GS mRNA levels. Data were expressed as mean ± SEM (n=12), **p*<0.05 *vs.* control, ***p*<0.01 *vs.* control.

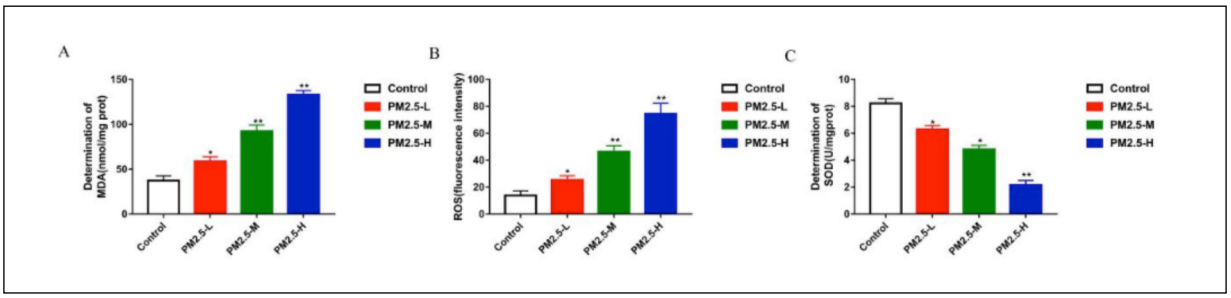

Figure 8. Effect of PM2.5 on oxidative stress response in mouse liver. **A,** Content analysis of MDA. **B,** Content analysis of ROS. C. Content analysis of SOD. Data were expressed as mean ± SEM (n = 12), *p<0.05 *vs.* control, **p<0.01 *vs.* control.

(p<0.05). Compared with the control group, the expression of MDA in the PM2.5 group was significantly increased (Figure 8A), the difference was statistically significant (p<0.05). When compared to the control group, the ROS expression in the PM2.5 group was much higher (Figure 8B), and the difference was statistically significant. These findings imply that PM2.5 affects the oxidative stress response in the liver of mice (p<0.05).

Discussion

At present, the issue of environmental pollution has attracted much attention, and relevant studies[23,24] have proved that the occurrence of many diseases is closely associated with air pollution. A large number of epidemiological and toxicological data show that PM2.5, as the primary harmful air pollutant, can cause inflammation of the respiratory system and even lead to diseases in the cardiovascular system and immune system, and then induce cancer of various types, posing a severe threat to human health. Most of the components of PM2.5 contain a variety of mutagenic and carcinogenic substances such as polycyclic aromatic hydrocarbons and heavy metal ions. After inhalation, PM2.5 can not only deposit in the alveoli and enter the blood circulation, but even reach other organs through lung ventilation, which will cause damage to extra-pulmonary organs and ultimately damage human health. PM2.5 is particularly associated with lung dysfunction, systemic inflammation and oxidative stress, metabolic syndrome, and cardiovascular disease[25-27]. Short-term exposure to PM2.5 can cause acute lung inflammation damage in BALB/c mice[28], which suggests PM2.5 entering the lungs will eventually damage organs other than lungs. In addition, PM2.5 has a direct and profound effect on the liver, the main organ for detoxification and metabolism. Multiple studies[29-30] have shown that exposure to PM2.5 induces lipid accumulation, oxidative stress, insulin resistance, and inflammatory responses in the liver. At present, a large number of studies[31-33] have shown that PM2.5 is harmful to the liver in all aspects. Therefore, it is of certain theoretical significance to study whether PM2.5 has carcinogenic and mutagenic effects on liver cells. Hence, this study explored whether the liver will be affected by PM2.5 and the mechanism of injury. The BALB/c mouse model was utilized to investigate hepatic inflammation and oxidative stress-induced liver damage caused by short-term PM2.5 exposure.

The data of organ coefficients of experimental animals is an important basic index for biomedical research. It is not only useful for directing the normal physiological condition and food management of animals, but it is also an essential reference index for non-clinical pharmacological research. As a consequence, we investigated the organ coefficients of the mice in each experimental group, and the findings indicated that the organ coefficients of the PM2.5-L, PM2.5-M, and PM2.5-H groups rose in varying degrees, showing that PM2.5 can induce liver disease in mice. These findings suggest that PM2.5 may be linked to liver disease.

In the experiment, histopathology can intuitively show the damage to the organs. Therefore, we performed HE staining and TUNEL staining to reflect the changes in mouse liver. Compared with the control group, the mice in the PM2.5-exposed group had hepatic cell edema and cytoplasmic pathological changes, and apoptosis of the

cells was observed. Moreover, as the concentration of PM2.5 rose, the damage was increasingly severe, especially in the liver of the PM2.5-H group, and a large number of cells showed apoptosis, which indicate that PM2.5 caused apoptosis in the liver tissue and aggravated the degree of liver tissue damage. These data indicate that PM2.5 induces liver damage in mice, providing new evidence for the effect of PM2.5 on human health. It is speculated that PM2.5-induced apoptosis may be correlative with the promotion of oncogenes and apoptosis-related genes. In addition, some studies[34,35] have shown that the toxic effect of PM2.5 is associated with the disorders of amino acid metabolism, lipid metabolism, energy metabolism, and oxidative stress in mice, which indicate that PM2.5 exposure gave rise to the changes of small molecule metabolites in mice or their changes over time, and its toxic effect was exerted through the mutual changes of various metabolites.

ALT and AST are considered to be serological indicators with higher sensitivity to liver damage. As long as liver cell necrosis reaches 1%, blood transaminase activity can be doubled. Particularly, ALT is the most sensitive indicator of acute liver cell damage. Therefore, ALT and AST were tested. The analysis found that short-term exposure to PM2.5 can indeed cause liver damage. A study[36] has shown that exposure of experimental mice to PM2.5 can cause oxidative damage to the liver and other organs and increase the expression of hepatocyte inflammatory factors. As IL-6 and TNFα play a major role in many inflammatory cytokines, in this experiment, the levels of IL-6 and TNFα in the liver tissues of the PM2.5-exposed group and the control group were also tested. The analysis found that short-term exposure to PM2.5 can significantly cause liver molecular damage. These data indicate that PM2.5 affects inflammation in the liver of the mice, which is consistent with the previous report[37].

A study[38] has shown that after exposure to PM2.5 in experimental mice, the ROS generated in the body and the free radicals carried by PM2.5 can enter the liver with blood circulation, causing lipid peroxidation in liver cells and an imbalance in the oxidation and antioxidant systems of liver tissue, leading to a series of oxidative damages to liver cells or accelerated liver fibrosis. Exposure to PM2.5 can also induce liver fibrosis in the liver of mice and impair liver glucose metabolism[39]. Thus, we examined oxidative stress in mice. The SOD, MDA, and ROS indicators, expressions of the oxidative stress, revealed that PM2.5 would give rise to the up-regulation or down-regulation of oxidative stress indicators in varying degrees, which is consistent with previous research[40], demonstrating that PM2.5 can induce oxidative stress in the liver of mice. In addition, we also tested the liver glycogen and blood glucose levels in mice, and the results showed that the blood glucose in the PM2.5-L, PM2.5-M, and PM2.5-H groups increased, and the number of liver glycogens decreased, indicating PM2.5 can damage the liver's sugar metabolism. Our findings provide new evidence for the effect of PM2.5 on the metabolism in liver. The reason could be that external stimulation of the liver and brain led to changes in amino acid metabolism accompanied by changes in energy metabolism, thereby affecting the normal physiological functions of the liver and brain. Glycogen and glucose were decreased in each PM2.5-exposed group in the liver and brain of the mice, and glycerol and triglyceride in each group were significantly reduced. All of these indicate that after PM2.5 exposure in mice, the tricarboxylic acid cycle was disturbed, mitochondrial function was impaired, and adenosine triphosphate (ATP) generation was hindered, which in turn accelerated fat mobilization, so as to compensate for the deficiency of ATP by enhancing the glycolytic pathway, impaired ATP production, and then accelerated fat mobilization, compensates for ATP deficiency by enhancing the glycolytic pathway.

Since the *Nrf2/JNK* signaling pathway plays an important role in a variety of liver diseases, including inflammatory response, fibrosis, and even tumorigenesis[41-44], in this experiment, we also investigated the related molecules in the *Nrf2/JNK* signaling pathway. The expression levels of genes and proteins related to the *Nrf2/JNK* signaling pathway were detected, through which, we found that compared with the control group, the levels of Nrf2 in the PM2.5-L, PM2.5-M, and PM2.5-H groups were significantly increased, and the phosphorylation level of *JNK* increased, and the gene level of *INK* also increased. This suggests that the *Nrf2/JNK* signaling pathway is activated. Our findings provide new evidence of the mechanism by which PM2.5 causes liver injury by targeting at *Nrf2/JNK* signaling. *Nrf2/JNK* signaling may be just one of the downstream mechanisms of PM2.5 and other potential factors should be explored in future investigations.

Conclusions

Short-term PM2.5 exposure through systemic blood circulation can cause liver inflammation and liver damage mediated by oxidative stress in mice. Short-term exposure to atmospheric PM2.5 can induce the synthesis and degradation of hepatic ketone bodies as well as changes in the metabolic pathways of alanine, aspartic acid, glutamic acid, taurine, hypotaurine, and glutathione, which indicated that PM2.5 disturbs amino acid metabolism and oxidative stress in the liver. Among them, the activation of the *Nrf2/JNK* signaling pathway may be involved in liver inflammatory damage caused by PM2.5. The results of this study have certain theoretical significance and application value for further in-depth exploration into the health hazards of PM2.5 in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Data Availability

The datasets used during the present study are available from the corresponding author upon reasonable request.

Ethics Approval

This study was approved by the Animal Ethics Committee of Shanghai Tenth People's Hospital (No. SHDSYY-2020-2420).

Informed Consent

Not applicable.

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Authors' Contribution

Q. Zhang drafted and revised the manuscript. H.-Y. Zhang, X.-Q. Yu, Z.-J. Cui, Z.-W. Lv conceived and designed this article, in charge of syntax modification and revision of the manuscript. All the authors have read and agreed to the final version manuscript.

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