ROLE OF INTESTINAL FLORA DYSBIOSIS IN PM2.5 EXPOSURE-MEDIATED NEUROTOXICITY IN RATS AND AURICULARIAN POLYSACCHARIDE INTERVENTION

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ABSTRACT

Objective*: To implore the role of gut flora in PM2.5 exposure inducing neurotoxicity of rats.*

Methods: *64 SD rats were randomly divided into control group, PM2.5-exposed group , low-dose intervention group (intervention A) and a high-dose intervention group (intervention B).We examined their neurobehavioral changes and gathered the blood serum and brain tissue, ELISA was used to determined the protein contents of IL-1β, IL-10, IL-17, BDNF, and TGF-β, Real-time PCR was applied to measure the mRNA expression of IL-17, IL-1β and TNF-α in the hippocampus. High-throughput sequencing quantified intestinal flora composition from fecal samples.*

Results: *PM2.5 exposure markedly disrupted neurocognitive function that was partially reversed by AP treatment. Compared with the control group, of IL-1β and TNF-α mRNA expression in PM2.5 exposed group were significantly increased. In addition, the auricularian polysaccharide intervention group showed a significant decrease in terms for IL-1β and TNF-α mRNA expression . Meanwhile, the content of IL-17, IL-1β and TNF-α in PM2.5 exposure group increased than that of the control group. Content of IL-17 and TNF-α decreased in auricularian polysaccharide intervention group relatived to PM2.5 exposure group, however, IL-1β had no significant differation compared to PM2.5 exposerd group .Compared with the control group, the content of IL-17 in the PM2.5 exposure group was significantly increased and the IL-10 content showed a decreased trend in expression. Following auricularian polysaccharide intervention, the content of IL-17 decreased, and the expression of IL-10 and TNF-α increased in two treatment groups; High-throughput sequencing showed that the abundance index (chao1) and diversity index (Shannon) of intestinal flora were higher in controls relative to PM2.5 exposed animals. All reads were clustered into 1506 OTUs (operational taxonomic units). The dominant community of taxonomic compositions at the phylum level were firmicutes, bacteroidetes, actinobactric, proteobacteria, Verrucomicrobia and tenerientes, actinobactric, proteobacteria and tenerientes all increasedin the PM2.5 exposed group relatived to the control group, firmicutes, and bacteroidetes declined. Bacteroidetes in the auricularian polysaccharide intervention group was four-fold increase as compared with PM2.5 exposure group.Clustering analysis showed that the primary genus were adlercreutzia, leacobacter and oscillospira in PM2.5 exposed group,acinetobacter,roseburia,bacteroides, fimicutes, vibrio, chloroflexi, and akkermansiaand in the auricularian polysaccharide intervention groups ,Gut microbial composition was significantly disrupted by PM2.5 exposure.*

Conclusion: *PM2.5 exposure disrupted neurocognitive capabilities that depended on altered intestinal flora and inflammatory mediator. Early auricularian polysaccharide intervention retarded to decrease CNS injury following PM2.5 exposure.*

Keywords: *PM2.5 exposure, central nervous system (CNS), auricularian polysaccharide intervention, intestinal flora.*

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Introduction

Atmospheric particulate matter (PM) exposure, and particularly fine PM (PM2.5), which is defined as PM comprising a variety of recondensed and aggregated ambient atmospheric mixtures of gases, heavy metals and semi-volatile organic compounds, presents as a significant global health challenge[1, 2]. Several studies have indicated that PM2.5 exposure impaired major pulmonary and coronary systems, and provokes major health effects including those typical of respiratory and cardiovascular diseases(CVD)[3, 4]. Studies have also shown that PM2.5 exposure stimulates inflammatory reactions and generates inflammatory injury of the blood vessels and even central nervous system (CNS)[2, 5].

Impacts of PM2.5 exposure on the CNS has received considerable focus and research efforts[6, 7]. PM2.5 exposure affects the integrity of the CNS and contributes to the development of neurodegenerative diseases via pathways dependent on inflammation, oxidative stress, and microglial activation[8, 9], with manifestations evident by two of the most prevalent neurodegenerative diseases Alzheimer's diseases (AD) and Parkinson's diseases (PD)[10, 11]. Furthermore, emerging data supports inflammation of the brain playing a key role in the pathogenesis of affected disorders and impaired cognition ablity[12, 13].

Studies have previously indicated that the brain-gut-microbiotic axis played an important role in the development and observed processes in neurodegenerative diseases through a bidirectional homeostatic route of communication[14, 15]. The intestinal microbiota is highly complex and is thought to interact with the host via immunological, neuronal, and endocrine-mediated pathways. The dysbiosis of intestinal flora is considered a crucial factor in potentially promoting the development of autism, depression, AD and PD[16-18]. additionally, the presence of clostridia and bacteriodetes were prevalent in many of neurodegeneration disease. High counts of enterobacteriaceae and low counts of prevotellaceae were seen in PD patients.

Moreover, altered constitution of pathogenic bacteria in the gut further increased barrier permeability by synthesis or secretion of endotoxins and inflammatory mediators[19]. Much data support that cytokines and chemokines are involved in the inflammatory reactions, the pathogenesis of affecting disorders and impairing cognition following PM2.5 exposure, which collectively are thought to induce nervous system damage[20]. The proinflammatory cytokines IL-1-β and TNF-α both play important roles in neurodegenerative diseases[21-23]. IL-10, which is recognized as an anti-inflammatory cytokine with immunosuppressive effects, is regarded as a potential therapeutic target in neuroimmune disease[24-26].

In addition, several studies have demonstrated that IL-10 inhibited microglial secretion of TNF-α, which is elevated in the setting of acute brain injury[25, 27] . Becausce of beneficial effects of IL-10 in the CNS, Some studies expect a modulated diet to play an important role by stimulating IL-10 and suppressing TNF-α expression in nervous system injury. However, no studies have yet reported that the role of the intestinal microflora in nervous system damage following PM2.5 exposure. Many subjects were studied to improve the condition of intestinal flora dysbiosis, including the application of probiotics[28]. Probiotics are fermentable carbohydrates and dietary fiber that are thought to form a biological barrier in the gastro-intestinal tract with the intent of excluding and restraining harmful pathogenic bacteria by enhancing the growth of specific beneficial bacteria found in the gut[29]. Probiotics not only alter the intestinal microbiota, they are also thought to strengthen intestinal tight junction integrity and to decrease blood endotoxemia that is caused by secretion of endotoxin/lipopolysaccharide or LPS[30].

Prebiotics could also protect animals against inflammation. Moreover, auricularian polysaccharide, as a representative therapeutic article was studied in the current report. Auricularian polysaccharide was extracted from auricularian, and is made up of several components of polysaccharide[31]. Studies have indicated that auricularian polysaccharide treatment could improve immune function[32-34]. In the current study, we determined whether or not auricularian polysaccharide could reduce inflammation and affect the intestinal microbiota following PM2.5 exposure. We expected that auricularian polysaccharide could modulate the intestinal microflora and that it might indeed represent a potential probiotic agent in the treatment of PM2.5-mediated damage.

Despite the important observations with regard particular bacteria-derived products that affected blood-brain-barrier (BBB) development and maintenance[16], the exact mechanisms thought to be involved remain poorly defined. In recent years, studies have attempted to address the role played by the CNS pathway and have explored the scope of influence imparted by gut microbiota on the CNS and subsequent changes in neuroethology[35-37].

In the present study, we applied high-throughput pyrophosphate sequencing to analyze changes in the intestinal flora constitution in rats exposed to PM2.5 and the potential benefits of auricularian polysaccharide intervention. We further explored the roles played by intestinal microflora following PM2.5 exposure and induced nervous tissue damage in anticipation of a therapeutically useful mode of prevention and treatment following ambient fine PM atmospheric pollutant exposure.

Materials and methods

Experimental animals

All experimental animals were approved by the local Medical Experimental Animal Administrative Committee of North China University of Science

and Technology. Sixty-four SD rats, weighing an average of 220-240g, were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd.

All rats were acclimated with free feeding and drinking for one week prior to the start of our described studies.

Preparation of PM2.5 working suspension

The ambient atmospheric PM2.5 particulates were provided by the environmental monitoring center of Tangshan city and preserved at 4 °C before being diluted for this experiment at the required dosing concentrations. The stock solution (4 mg/ml) was preceded by treatment for 30 min under ultrasonic oscillation. The particles were then sterilized by autoclaving under standard conditions. Each working solution (4ml/kg-body weight) was freshly made by diluting the stock solution with sterile saline.

Treatment of animals

The acclimated rats were randomly divided into four groups comprising the following: control group (n=16), PM2.5 exposed group (n=16), low-dose intervention group (n=16) and a high-dose intervention group (n=16). Rats in the PM2.5 exposure group were treated with a PM2.5 working solution (4ml/kg-body weight) through tracheal perfusion, once per week for eight weeks. Rats in the low- and high-dose intervention groups were given 100 and 200 mg/kg auricularian polysaccharide by gavage following PM2.5 treatment.

Morris water maze test

This test was aimed at detecting memory capacity and spatial orientation. The test included a navigation test and crossing a platform.

From the first to the third day, the place navigation test was determined. Rats of each group were observed to record time taken to find the hidden platform. On the fourth day, the hidden platform was removed, and the escape latency of animal was assessed in any quadrant of the water pool to test the ability of the rat to escape within 120s.

New object recognition test

This test determines memory capacity of rats through flexible transformation of the new object including the shape or magnitude of the object. The program consists basically of adaptation, familiarity and actual test. First, the rats are placed into the experimental device in the absence of any object, and then enable them to freely explore their environment in order to adapt to it and minimize the irritability of the animal. Then, two identical objects (A1 and A2) are placed into the bottom , and the rats are placed into the experimental device with their backs positioned toward two objects in the experiment.

After a time interval, one of the two identical objects is changed by another object. Both test objects are respectively named with the familiar object (A3) and the novel one (B). The exploring periods and time were recorded . Preference for the original object by the experimental animals can be used to quantify the index of the "discrimination index," which is generally expressed as D2 (D2), using the following equation: D2 = (N.F)/(N + F); where "N" assesses the animals in their exploration of the novel object when tasked, and "F" showed the test period for animals to become familiar with objects over a measured or explored time period. The "discrimination index" was used to determine the behavior of animals when challenged across different levels of exploration.

Elevated plus maze test

Elevated plus maze was used to assess anxiety-like behaviors. At the beginning of the experiment, rat movements from the center of the maze over a period of five minutes were recorded. Observational indices include the number entering with the open arm (two former melons enter the arm), the time rats stayed in place under conditions of the open arms, the number of rats entering with closed arms, and the time rats stayed in placed under conditions of the closed arm. The ratio of the number of rats entering with open arms and times, and the ratio of the staying in place time with the open arms, and the total elapsed time were calculated.

Enzyme-Linked Immunosorbent Assays (ELISA)

The protein contents of IL-1β, IL-10, IL-17, BDNF, and TGF-β in both the brain tissue and blood serum were determined according to the manufacturer's instructions (Neobioscience). Detection limits for IL-1β, IL-10, IL-17, BDNF and TGF-β were respectively 80-50, 000 pg/ml, 15.6-1000 pg/ml, 1.6-100 pg/ml, 80-16, 000 pg/ml, and 16-2000 pg/ml.

Real-Time PCR

RNA extraction was carried out according to the manufacturer's protocol. Samples were reverse transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems) with random

primers. The levels of mRNA encoding Atp7a and Atp7b were quantified using PCR. In short, the total RNA was isolated from the hippocampus and cerebral cortex by using TRIzol reagent. An aliquot of RNA (20 ug) was reverse transcribed into cDNA by using the Bio-Rad iScript cDNA synthesis kit. The iTaq Universal SYBR Green Supermix (Bio-Rad) was used for real-time PCR analyses.

The amplification was run in the CFX Connect Real-Time PCR detection system (Bio-Rad) with an initial 3 min denaturation at 95°C; the amplification program was followed by 40 cycles of 30s denaturation at 95°C, 10s gradient at 55–65°C and 30s extension at 72°C. A dissociation curve was used to verify that the majority of the detected fluorescence was attributed to the labeling of specific PCR products, and to verify the absence of primer dimers and sample contamination. Each qPCR reaction was run in triplicate. Relative mRNA expression ratios between groups were calculated using the Ct formulation where Ct is the threshold cycle time value. The Ct values of interested genes were first normalized with that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same sample to obtain the Ct values, and then the relative ratios between the control and treatment groups were calculated and expressed as relative gene expression by setting the control as 100 percent. The amplification efficiencies of targeted genes and the internal reference were examined by determining the variations in Ct with a series of control template dilutions (Table 1).

IL-1β	5'GCAGTGTCACTCATTGTGGC3'	5'AAGGTGCTTGGGTCCTCATC3'
TNF-α	5'AAGGGAATTGTGGCTCTGGG3'	5'ACTTCAGCGTCTCGTGTGTT3'
BDNF	5'GCATACAGCCAGATACTAGAGC3'	5'TCCCCACCTCCATCTAGACC3'
GAPDH	5'AGGTCGGTGTGAACGGATTTG 3'	5'GGGGTCGTTGATGGCAACA3'

Table 1: The primers used for real-time PCR.

Analysis of intestinal flora

Fresh fecal samples were collected in the rats under biological safety cabinets and immediately stored at -80°C. Samples were transferred to the test laboratory within 24h in a bucket filled with dry ice. High-throughput sequencing was applied to determine fecal intestinal flora in the 16s-rRNA gene sequences of the V4 area.

The key steps included DNA extraction and detection in the macro genomic group, PCR amplification (primer: 520F AYTGGGYDTAAAGNG and 802R TACNVGGG TATCT AATCC), PCR product quantitation, and samples mixed (using the Quant-iT PicoGreen dsDNA Assay Kit for PCR product quantitation using a microplate reader (BioTek FLx800). Next, samples were mixed in accordance with the needed data volume for each sample. We used the Illumina TruSeq Nano DNA LT Library Prep Kit to build the library, and carried out Promega QuantiFluor with the Quant-iT PicoGreen dsDNA Assay Kit to quantify the library. We also used the MiSeq Reagent Kit V3 to conduct RNA-seq analysis.

Application of clustering, principal component and least squares analyses, enabled us to investigate the changes in intestinal flora composition and the correlation between the gut microbiota and inflammatory factors.

Statistical Analysis

All studied data are expressed as the mean ± SD, and assessed by one-way analysis of variance (ANOVA) to determine statistical significance. Statistical analysis was performed with GraphPad Prism 7.0 (GraphPad Software, Inc.). A value of P less than 0.05 was considered statistically significant.

Results

Auricularian polysaccharide retards to decreasein cognitive and memory capabilities of rats following PM2.5 exposure

In the current study, the Morris water maze, the new object recognition test, and the elevated plus maze test were applied to measure cognition and memory ability in PM2.5 exposed rats as well as auricularian polysaccharide intervention rats.

The escape latency at the third day of the PM2.5 exposed group was significantly longer than that of the control group, and the escape latency in the auricularian polysaccharide intervention groups declined as compared to the PM2.5 exposed group ($^{ab}P<0.05$; Figure 1a). The frequency of crossing the platform in the PM2.5 group of rats was decreased compared to the control group, and the auricularian polysaccharide intervention groups showed a greater frequency of platform crossing compared the PM2.5 group (abP<0.05; Figure 1b).

The new object recognition experiment was applied to analyze the exploration ability of PM2.5 exposed rats. The time of staying on exploring a new object significantly declined in the PM2.5 exposed groups relative to the control group and the auricularian polysaccharide intervention group (Figure 1c). Elevated plus-maze test was used to test the anxiety state of the rat following PM2.5 exposure. the percent

of the open arm time and total time were significantly lower than that of the control group. In addition, the percent of the open arm time to total time of the auricularian polysaccharide intervention groups was longer than PM2.5 exposure group (P<0.05); Figure 1d-e). Brain-derived neurotrophic factor (BDNF) plays an important role in the development and function of the CNS.

We found that mRNA expression of BDNF of hippocampus in the PM2.5 exposed group showed a significant decrease compared with the control. Further, BDNF mRNA expression was increased in the auricularian polysaccharide intervention group than that of PM2.5 exposure group (Figure 1f-g).

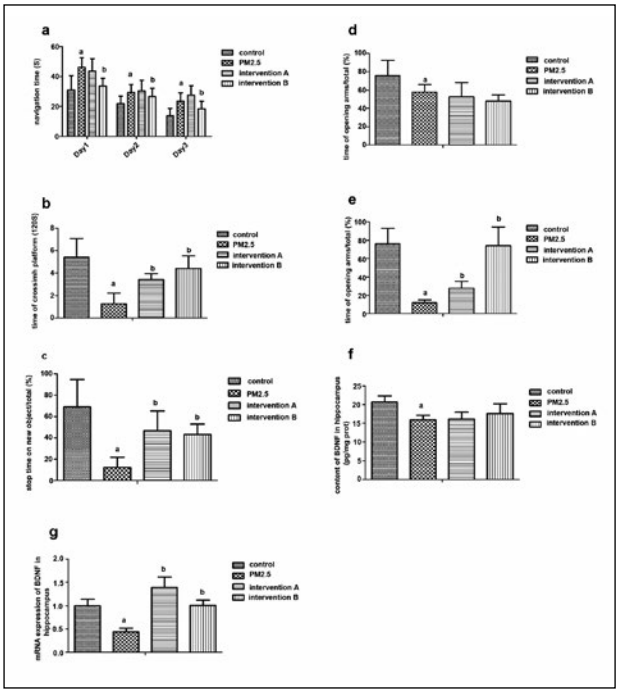


Figure 1: Neurobehavioral changes of PM2.5 exposed rats and auricularian polysaccharide intervention."a" showed the differation between control group and PM2.5 exposed group,"b" showed the differation between PM2.5 exposed group and auricularian polysaccharide intervention A or auricularian polysaccharide B group.
Compared with control group, [a]P<0.001; compared with PM2.5 exposure group, [b]P<0.001; n (16).

The change in mRNA and protein expressions of the inflammatory cytokines IL-17, IL-1β, and TNF-α in the hippocampus of rats following PM2.5 exposure and auricularian polysaccharide intervention

Real-time PCR was applied to measure the mRNA expression of IL-17, IL-1β and TNF-α in the hippocampus. Compared with the control group, of IL-1β and TNF-α mRNA expression in PM2.5 exposed group were significantly increased (P<0.001). In addition, the auricularian polysaccharide intervention group showed a significant decrease in terms for IL-1β and TNF- α mRNA expression (P<0.001) (Figure 2a). Meanwhile, the content of IL-17, IL-1β and TNF-α in PM2.5 exposure group were all significantly increased than that of the control group (P<0.05). And IL-17 and TNF-α content were all decreased in auricularian polysaccharide intervention group than those of PM2.5 exposure group (P<0.05), however, IL-1β had no significant differation compared to PM2.5 exposerd group (Figure 2b).

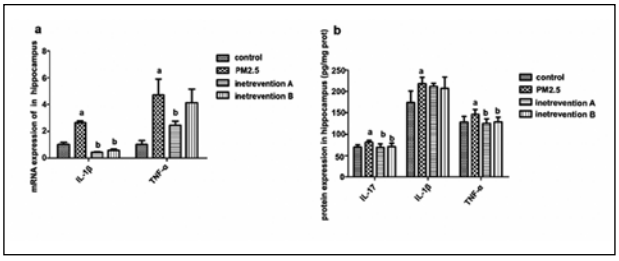


Figure 2: The mRNA expression and protein content of related inflammatory cytokines in the hippocampus following auricularian polysaccharide intervention. Data are expressed as mean±SD.
Compared with control group, [a]P<0.001; compared with PM2.5 exposed group, [b]P<0.001; n=16.

Alteration of inflammatory factor IL-17, IL-10, TGF-β in serum of rats following PM2.5 exposure and Auricularian polysaccharide intervention

Compared with the control group, the content of IL-17 in the PM2.5 exposure group was significantly increased and the IL-10 content showed a decreased trend in expression (P<0.05) (Figure 3A-B). Following auricularian polysaccharide intervention, the content of the inflammatory factor IL-17 decreased, and the expression of IL-10 and TNF-β increased in two treatment groups (P<0.05); (Figure 3A-C).

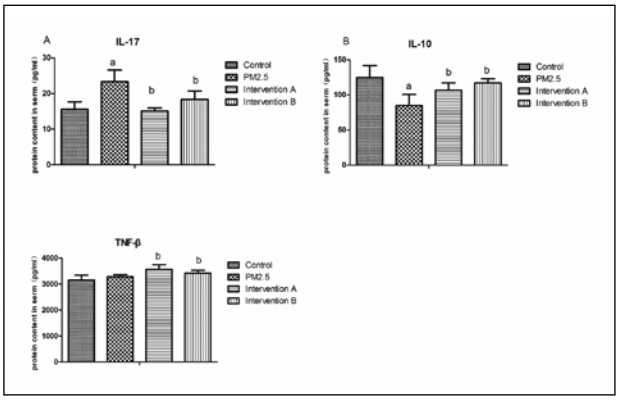
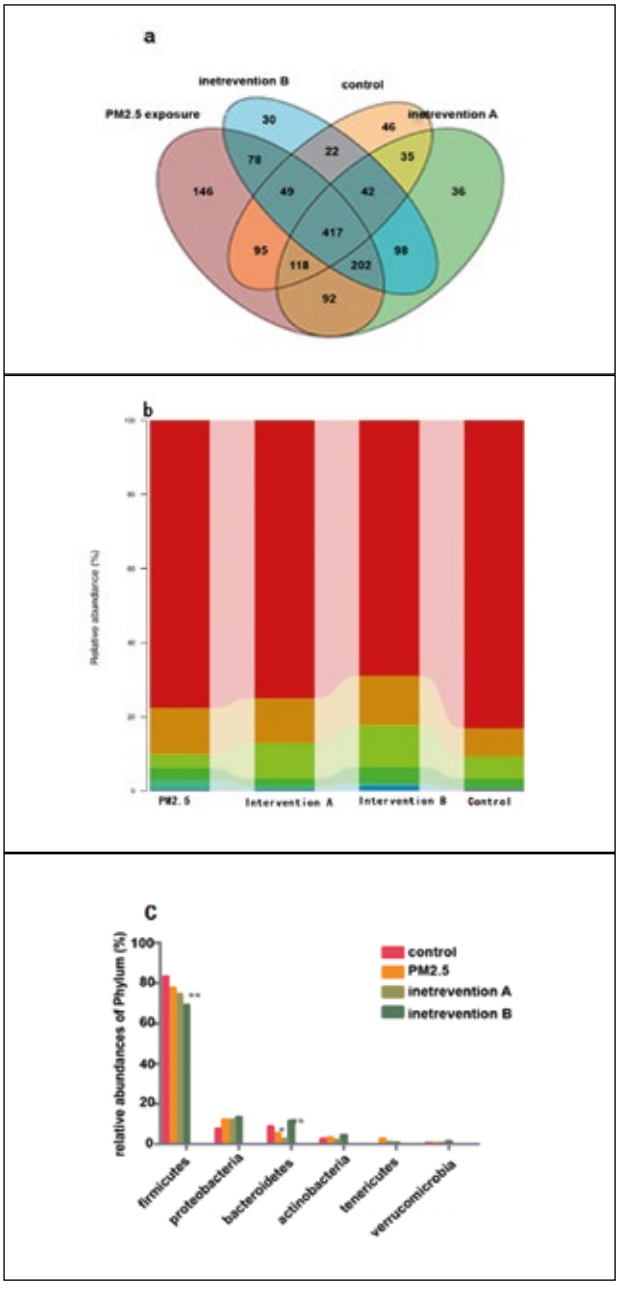


Figure 3: Protein expression of inflammatory cytokines IL-17, IL-10, TGF-β in serum of rats following PM2.5 exposure and Auricularian polysaccharide intervention.
Compared with control group, [a]P<0.001; compared with PM2.5 exposure group, [b]P<0.001; n=16.

Auricularian polysaccharide modulates intestinal microflora dysbiosis of rats following PM2.5 exposure

High-throughput sequencing was applied to determinate fecal intestinal flora. Bacterial 16s rRNA V3-V5 sequences were amplified from fresh fecal samples of all tested SD male rats. In total, All reads were clustered into 1506 OTUs (operational taxonomic units) at 97% sequence similarity. A Venn diagram showed that 1342 OTUs were detected in the control group and PM2.5 exposure group, 1408 OTUs in the PM2.5 exposure group and intervention A, 1389 OTUs in the PM2.5 exposure group and intervention B, while 146 and 66 OTUs were unique to rats following PM2.5 eposure and auricularian polysaccharide intervention (Figure 4a).



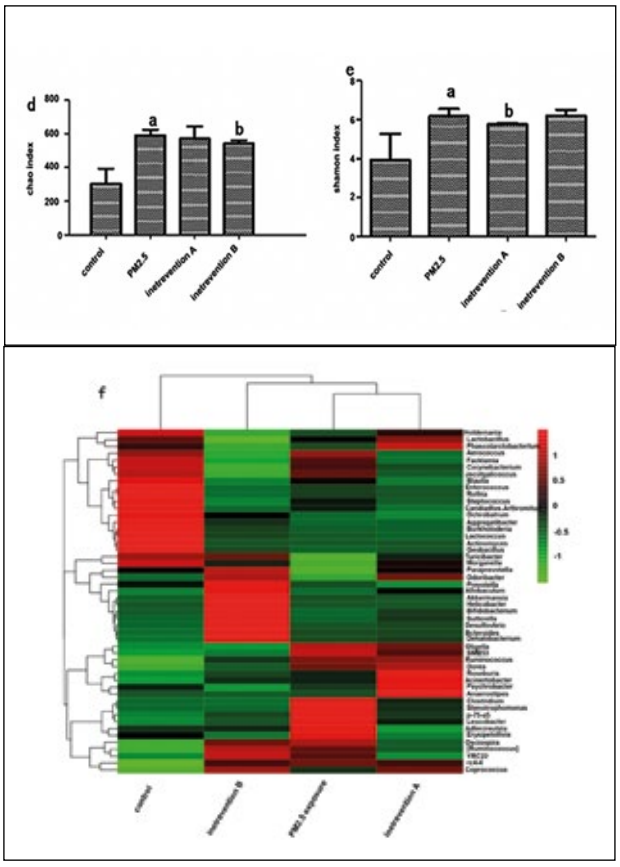


Figure 4: (**a**) Venn diagram; (**b-c**) Dominant community of taxonomic compositions at the phylum level; (**d-e**) reflecting the richness estimators"Chao1" and the diversity indices "Shannon"; (**f**) Clustering analysis of community of intestinal flora composition .

According to the OTUs of intestinal flora,the dominant community of taxonomic compositions at the phylum level were firmicutes, bacteroidetes, actinobactric, proteobacteria, Verrucomicrobia and tenerientes (Figure 4b).

Compared to the control group, actinobactric, proteobacteria and tenerientes in the PM2.5 exposed group all increased; However, firmicutes, and bacteroidetes declined ($P<0.05$; Figure 4c).

Bacteroidetes in the auricularian polysaccharide intervention group was four-fold increase as compared with PM2.5 exposure group (Figure 4c).

In addition,the richness estimators Chao1 and the diversity indices Shannon in the PM2.5 exposed group were all higher than that found in the control group ($P<0.05$), Furthermore, compared with PM2.5 exposure group, the richness of gut flora in the auricularian polysaccharide intervention B group displayed changes,however, the diversity of gut flora began to change in intervention A group (Figure 4d-e).

Clustering analysis of community intestinal flora composition showed that the primary genus were

adlercreutzia, leacobacter and oscillospira in PM2.5 exposed group, and in the auricularian polysaccharide intervention groups, the genus were primarily acinetobacter, roseburia, bacteroides, fimicutes, vibrio, chloroflexi, and akkermansia (Figure 4f).

Discussion

Disorders of intestinal flora have played important roles in nervous damage[38, 39]. There was also an anatomical association demonstrated between the intestine and the central nervous system in previously published work[40]. The function of the intestinal tract is regulated through both the CNS and gut microbiome[41]. Under the physical condition, the intestinal microflora are generally stable, and the gut barrier can protect against the translocation of gastrointestinal bacteria and some bacterial-derived products from trafficking to the brain and the peripheral blood system[42]. Studies showed that the composition of gastrobacteria was influenced by emotional and physiologic stress as well environmental pollutants[43-45].

In this study, 16S rRNA gene sequencing was applied to investigate the community taxonomic composition at the phylum to genus levels of the gut microbiota. According to the OTUs of intestinal microflora of all tested SD male rats , the community taxonomic composition at the phylum level was primarily composed of firmicutes, bacteroidetes, actinobactric, proteobacteria, Verrucomicrobia and tenerientes. These bacteria can produce short-chain fatty acids (SCFAs), especially by clostridium species of the phylum firmicutes. Butyric acid was one of the SCFAs that was identified, which can enhance the barrier function of intestinal mucosa to prevent the migration of gut bacteria into the peripheral blood[46]. Butyrate was previously shown to play an important role in modulating the neuroinflammatory pathway, particularly its potential anti-inflammatory properties[47].

the taxonomic abundance distributions were significantly different among groups as defined by the abundance index“chao1”and the diversity index “shannon”. Clustering analysis of community compositions showed that the primary flora at the genus level in PM2.5 exposed rats were adlercreutzia, leacobacter, oscillospira, and there were primarily acinetobacter, roseburia, bacteroides, fimicutes, vibrio, chloroflexi, and akkermansia found in the intestinal flora of rats treated with auricularian polysaccharide intervention. Alterations in the intestinal microbiota were associated with key inflammatory factors, and decreased communities of fimicutes, roseburia, bacteroides, vibrio and chloroflexi might result in high expression of the inflammatory cytokines IL-1β and TNF-α. These findings suggest a marked effect of the gut microbiota on overall composition.These data would be transformed into specific therapies and useful in treating or preventing PM2.5-induced CNS damage.

Previous studies showed that PM2.5 exposure provoked nervous system damage[9, 48]. Our results showed that the cognitive function of rats declined as a consequence of exposure to PM2.5, which was consistent with previous studies[49]. PM 2.5 exposure was associated with cognitive decline or acceleration of AD progression[50, 51]. In this current study, the Morris water maze study, the elevated plus-maze study and the new object recognition experiment were applied to determine cognitive function. Observations from these studies showed that cognitive function declined in PM2.5 exposed rats. Many studies have indicated that BDNF plays an important role in nervous tissue damage[52] . In addition, BDNF was shown to be a protective factor, and that its overexpression could prevent neurocognitive deficits[53].

In the present study, auricularian polysaccharide intervention was applied to PM2.5 exposed rats. The role of inflammation in the progression of PM2.5-induced CNS damage was also previously reported by others[54, 55]. Cunningham[56] indicated that inflammation induced acute behavioral and cognitive changes and accelerated disease progression. Inflammatory cytokines such as IL-17, IL-10, IL-1β, TNF-α, and TGF-β played an important role in nervous tissue damage. Moreover, the pro-inflammatory cytokine IL-17 can induce the expression of a variety of chemokines and cytokines, and participates in immune responses and inflammatory reactions[57, 58]. By contrast, IL-10 is an anti-inflammatory cytokine and was previously shown to inhibit the synthesis of cytokines, including IL-2 and IL-3[59], and plays many roles in the control of inflammation, function, and homeostasis of the gastro-intestinal immune system[60]. In the current study, we have clearly shown that the expression levels of IL-17, IL-10, and TGF-β all showed rapid changes in the hippocampus. There were also significant increases in the expression levels of the inflammatory cytokines IL-17, IL-1β and TNF-α as well as decreased levels of IL-10 in the PM2.5 exposed group of animals.

Moreover, the auricularian polysaccharide intervention group of animals showed a significant de-

crease in the functional expression of the inflammatory cytokines IL-17, IL-1β and TNF-α and increases in the expression of IL-10. Others have shown that neuroinflammation is involved in multiple neurodegenerative diseases including AD, PD and multiple sclerosis or MS[61, 62]. Intestinal microflora were also shown to be associated with many neurodegenerative diseases, and that changes to the gut microbiota might result in increased or decreased inflammatory factors[63].

Conclusion

Auricularian polysaccharide intervention significantly increased the diversity of the microbiota, and affected the gene expression of inflammatory cytokines; The findings suggest that auricularian polysaccharide may have some certain interventional qualities and effects with regard the setting of nervous tissue injury following ambient airborne PM2.5 triggering by the inhalational route of exposure.

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Data Availability:
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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