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*Article*

# Air-Pollutant Particulate Matter 2.5 (PM<sub>2.5</sub>)-induced Inflammation and Oxidative Stress in Diseases: Possible Therapeutic Approaches

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**Abstract:** Today, air pollution is the greatest threat to organismal healthspan. The environment of our planet earth, the habitat of over eight billion humans and estimated twenty billion billions other animals, is contaminated with a wide variety of pollutants. Unfortunately, humans, out of billions and billions of living organisms on earth, are solely responsible for polluting the environment through emitting pollutants like particulate matter from industry, fuel engine vehicles, biomass combustion, toxic fumes from blasting, and wildfire. In the modern world, human-caused air pollutants induce massive oxidative stress and inflammation, the major contributors in initiation and progression of many diseases including pulmonary, cardiovascular, renal, hepatic, reproductive, neurological, mental, and accelerated biological aging. The provocative question is the following: how can we solve this human-created problem? As it is not realistic to clean the environment at once from human-caused pollution, initiatives have been undertaken to develop novel therapeutic approaches to control air-pollutant-induced oxidative stress and inflammation to protect humans from pollution-induced devastating diseases. In this article, I discuss the key findings of numerous recent preclinical studies documenting first, the role of air pollutant PM<sub>2.5</sub> in augmentation of inflammation, oxidative stress, and associated diseases; and second, the efficacies of different natural and synthetic compounds in amelioration of PM<sub>2.5</sub>-induced oxidative stress, inflammation, pyroptosis, and associated pathologies.

**Keywords:** Air pollution; Particulate Matter<sub>2.5</sub>; Inflammation; Nlrp3; Oxidative Stress; Nrf2; PAI-1; Aging

## 1. Introduction

As the fauna and flora in every corner of the earth are interdependent for survival, it is pivotal to keep the air and water quality safe for food and shelter of all living organisms and maintenance of healthy ecosystem. While good air quality of our habitat has immense impact on our healthy life, air pollution is the greatest risk factor for development of numerous diseases resulting in accelerated aging and shortened healthspan [1]. It is noteworthy that initiation of every disease stems from impaired inflammation and oxidative stress responses. The key events of inflammation in response to stress, injury, and infection are vascular dysfunction, infiltration of mononuclear immune cells including monocytes and macrophages, inflammatory cytokine storm, and activation of downstream inflammatory signaling. Importantly, inflammation is an essential response for healing in the early stage of injury or infection, and thus preserves tissue homeostasis. Furthermore, inflammatory cells also contribute to oxidative stress and impaired antioxidant system, another key early cellular response required to protect organisms from further vascular, cellular and tissue damage. However, persistent uncontrolled inflammation and oxidative stress in response to external or internal stressors lead to initiation and progression of numerous diseases due to impaired cellular physiology and tissue homeostasis [2]. It is noteworthy that one of the major igniters of massive inflammation and oxidative stress in the body is inhaled air pollutant. Unfortunately, human-caused air pollution is rapidly increasing in the modern world in diverse ways as follows: emitting fuel combustion from excessively growing number of vehicles, toxic fumes from blasting, wildfire smokes, smokes from

biomass burning, coal burning, crop residue burning, and factory released smokes. These are the key sources of elevated levels of particulate matter (PM), the major hazardous air pollutants in the environment. Recent survey showed that the levels of air pollutants are extremely high in the industrial belts and urban areas worldwide where billions of humans and other animals are exposing themselves to hazardous air pollutants, particulate matter daily for several hours. In recent years, the real time world's air pollution index exhibit that the air pollutant PM<sub>2.5</sub> levels of many highly populated cities in industrial belts exceeds >300-500 µg/cubic meter (m<sup>3</sup>) compared to standard <50 µg/m<sup>3</sup> [World's Air Pollution: Real-time Air Quality Index @ <https://waqi.info>; Current Air Quality @ <https://www.airnow.gov>]. It is well documented that both short-term and long-term exposure to PM<sub>2.5</sub> cause massive inflammation and oxidative stress in lungs and other organs. Both impaired inflammatory and oxidative stress pathways ignite the onset of numerous human diseases including chronic obstructive pulmonary disease, allergic rhinitis, vascular thrombosis, hypertension, arrhythmia, stroke, dementia, hepatic and renal diseases, abnormal childbirth, autism spectrum disorder, anxiety, infertility, cancer, and accelerated biological aging [3-10].

The first and foremost question is the following: how can we protect ourselves and other animals on earth from dangerous air pollutant-induced devastating diseases and shortened healthspan? There are two ways: obviously, the first choice is to diminish the human-caused environmental pollution; and the second possibility is to develop potential therapy to alleviate air pollutant-induced pathologies. The first choice is not realistic in this ultramodern human society within a brief period, but it is possible only through well-planned long-term global efforts. This positive notion is at least partially supported by the findings that the air pollutants were significantly reduced (40-50%) in numerous cities in the world during COVID-19 lockdown for a short period due to less emission of pollutant PM<sub>2.5</sub> from fuel combustion and factories [11-13]. The second choice is quite feasible, if we understand in-depth the air pollution exposure-induced deregulation of molecular and cellular events those contribute to persistent inflammation, oxidative stress, and development of multiple diseases. Last two decades, many *in vitro* and *in vivo* studies have been conducted to understand the effects of air pollutant exposure on cellular abnormality and organismal health and pathology. The purpose of this article is to discuss the significant findings on the induction of massive inflammation, oxidative stress, and initiation of disease development in response to PM<sub>2.5</sub> exposure using cellular and animal models. The promises of different therapeutic approaches using synthetic and natural compounds in amelioration of PM<sub>2.5</sub>-induced inflammation, oxidative stress, and multi-organ pathogenesis at the preclinical level are also discussed.

## 2. Air-Pollutant Particulate Matter (PM) and Its Mode of Action

Particulate Matter (PM) is the most hazardous air pollutant that holds a wide range of toxic substances including radon, sulfates, nitrates, benzene, polycyclic aromatic hydrocarbons, heavy metals like lead, cadmium, arsenic, chromium, barium, organic carbon, elemental carbon, and airborne bacteria. Based on published data, the composition of the PM varies in different cities in the world depending on the sources like generation from factory exhausts, vehicle fuel/diesel combustion, biomass burning, fumes from blasting, and wildfire, and the season of PM<sub>2.5</sub> collection [11-20]. The partial composition of Air pollutants collected in various parts of the world are published [13-15,17; also see NIST Certificate of Analysis, SRM 1649a, <https://tsapps.nist.gov/srmext/certificates/archives/>]. Based on its aerodynamic diameter, PM has been classified as coarse (10 µm or smaller in diameter PM<sub>10</sub>), fine (2.5 µm or smaller in diameter PM<sub>2.5</sub>), and ultrafine (0.1 µm or smaller in diameter PM<sub>0.1</sub>) [4,7]. Upon short-term or long-term inhalation, these original or chemically modified forms of fine particles are associated with induction of massive oxidative stress, inflammation, and development of pathologies. The elevated level of PM<sub>2.5</sub> in the atmosphere is the most hazardous risk factor to human health. While the acute harmful effects of PM<sub>2.5</sub> may be direct, involving rapid crossover from the lung epithelium into the circulation, the chronic effects of PM<sub>2.5</sub> involve generation of pulmonary oxidative stress, systemic inflammation, secretion of elevated levels of inflammatory cytokines and cellular dysfunction [21-24]. However, eventually, both direct and indirect effects of PM<sub>2.5</sub> ignite the onset of oxidative stress, inflammation, pyroptosis and progression of devastating pathologies including asthma, COPD, vascular thrombosis, organ fibrosis, heart failure, and accelerated biological aging.

### 3. PM<sub>2.5</sub> in Induction of Massive Inflammation and Oxidative Stress: Major Causes for the Initiation and Progression of Pathologies

In this section, I discuss the accumulated experimental evidence supporting the negative impact of air pollution PM<sub>2.5</sub> in ignition of massive inflammation, oxidative stress, and related pathogenesis.

#### 3.1. PM<sub>2.5</sub> Induces Inflammation and Oxidative Stress: Evidence from Gene Expression Profiling

Several unbiased global gene expression profiling provide evidence that exposure to air-pollutant PM<sub>2.5</sub> causes activation of inflammatory and oxidative stress pathways. For example, the gene expression profiling of control and PM<sub>2.5</sub>-exposed human bronchial epithelial cells (16HBE) by RNA seq analysis reveals that exposure to PM<sub>2.5</sub> (25 µg/cm<sup>2</sup>/for 24h) causes differential expression of 539 genes. Gene ontology analysis illustrates that PM<sub>2.5</sub> induces many genes involved in inflammation, oxidative stress, metabolism, xenobiotic stimuli, and cytokine-cytokine receptor interaction pathways. Additionally, exposure of cells to PM<sub>2.5</sub> is strongly associated with secretion of inflammatory cytokine IL-6 [25]. Histological and electron microscopy imaging data reveal that short term-exposer (24h and 48h) of mice to PM<sub>2.5</sub> (200 µg/mouse) causes an increased infiltration of neutrophils and macrophages in the lung tissues but not in liver compared to untreated animals [26]. Moreover, microarray analysis reveals that while, PM<sub>2.5</sub> exposer alters gene expression profiling of different pathways in lungs including chemokine signaling, HIF-1 signaling, inflammatory TNF- $\alpha$ , IL-17 signaling and cytokine-cytokine receptor interaction; in liver, PM<sub>2.5</sub> alters the expressions of numerous genes involved in metabolic signaling pathways including AMPK signaling, JAK-Stat signaling, cytokine-cytokine receptor and PPAR signaling [26]. Similarly, exposure of human and mouse macrophages to PM<sub>2.5</sub> (400-500 µg/ml) causes generation of oxidative stress (ROS), activation of inflammatory NF $\kappa$ B signaling, secretion of inflammatory cytokines IL-1 $\alpha$ , TNF- $\alpha$  and impaired phagocytosis, and thus disrupt inflammatory cell clearance by macrophages [27]. Furthermore, RNA seq analysis of RNA extracted from control and PM<sub>2.5</sub> (500 µg/ml for 24h) exposed PMA-primed THP-1 human macrophages reveal that expression of 1213 genes involved in different cellular pathways are deregulated by PM<sub>2.5</sub> including upregulation of IL-17, NF $\kappa$ B, TNF- $\alpha$ , and PPAR- $\alpha$  signaling pathways and downregulation of PI3K/AKT and cytokine-receptor interaction pathways [27]. Previously, we demonstrated that a short-term exposure (72h) to PM<sub>2.5</sub> (200µg/mouse) causes elevated levels of inflammatory markers Mac3, pStat3 and Vcam1 and apoptotic marker cleaved caspase 3 in murine lung and heart tissues [28]. Recently, we performed RNA seq analysis of RNA extracted from controls and PM<sub>2.5</sub> (200 µg/mouse) instilled (72h) murine lungs. The gene ontology analysis revealed that PM<sub>2.5</sub> significantly upregulated inflammatory pathway as shown by deregulation of many inflammatory genes including Nlrp3, IL-1 $\alpha$ , TNFrsf8, 9, 11a, 12a, 1b, and NF- $\kappa$ B2. In addition, many downregulated genes in response to PM<sub>2.5</sub> participate in metabolism (Ghosh AK et al. unpublished data). Collectively, these results on the impacts of air pollutant PM<sub>2.5</sub> on global gene expression profiling under different experimental milieus reveal that many commons signaling pathways are deregulated by PM<sub>2.5</sub> exposure including significant activation of inflammatory and oxidative stress pathways.

#### 3.2. PM<sub>2.5</sub>-Induced Inflammation, Oxidative Stress, and Allergic Rhinitis

It is well known that people with allergic rhinitis (AR) are more sensitive to air-pollutants. The impact of PM<sub>2.5</sub> in allergic airway inflammation has been studied using ovalbumin-induced AR mouse model [29]. Exposure of ovalbumin-induced AR mice to PM<sub>2.5</sub> (100 µg/mouse) causes augmented inflammation due to increased levels of inflammatory cytokines IL-4, IL-5, and IL-13 that eventually increases oxidative stress through malondialdehyde (MDA) synthesis. Furthermore, PM<sub>2.5</sub> exposure inhibits the level of Nrf2, the key regulator of antioxidant genes, in AR mice showing lack of protection of lungs from PM<sub>2.5</sub>-induced oxidative stress [29]. This is consistent with the observation that PM<sub>2.5</sub> (50µg/ml) reduces the levels of Nrf2 in cardiac fibroblasts [28]. A recent study showed that PM<sub>2.5</sub> (100 µg/mouse/day/for 30 days) significantly induces the infiltration of eosinophils in bronchoalveolar lavage fluid and inflammatory cells in the lung tissues of ovalbumin (OVA)-induced combined allergic rhinitis and asthma syndrome (CARAS) mouse model. While the levels of transcription factor GATA4, and Th2 and Th17 cytokines IL-4, IL-5, IL-13, and IL-17 are significantly increased compared to control, the levels of Th1 cytokines like IL-12 and IFN- $\gamma$  are significantly



decreased in nasal lavage fluid and broncho alveolar lavage fluid derived from CARAS/PM<sub>2.5</sub> mice compared to CARAS and control. Additionally, exposure to PM<sub>2.5</sub> leads to activation of NF- $\kappa$ B signaling in CARAS mouse model. These results confirm that PM<sub>2.5</sub> aggravates allergic inflammation by increasing the secretion of inflammatory cytokines [30]. In addition, the role of TLR2/TLR4 and MyD88 in PM<sub>2.5</sub>-induced (100  $\mu$ g/mouse/4 times in 2 weeks interval) worst inflammatory reaction in OVA-induced mouse model of asthma has been examined. While PM<sub>2.5</sub> exposure exacerbates OVA-induced lung inflammation or eosinophilia in wildtype mice as shown by increased levels of neutrophils, macrophages, and upregulation of IL-1 $\alpha$ , IL-5, IL-12, IL-13, chemokine KC in lungs, PM<sub>2.5</sub> fails to increase inflammation in TLR2 or TLR4 or MyD88 deficient mice [31]. Comparable results were obtained by Wang and colleagues [32] in an asthma mouse model exposed to PM<sub>2.5</sub>. Collectively, these results suggest that exposure to PM<sub>2.5</sub> aggravates allergic reaction where both inflammatory and oxidative stress pathways contribute to aggravated pulmonary symptoms in mouse model of AR and Asthma.

### 3.3. PM<sub>2.5</sub>-Induced Inflammation, Oxidative Stress, and Fibrogenesis

PM<sub>2.5</sub> exposure-induced inflammation and oxidative stress ignite matrix remodeling in the heart and lungs. Exposure to PM<sub>2.5</sub> (100  $\mu$ g/mouse/every 3rd day for total 9 days) induces the levels of secreted IL-17A, IL-1 $\alpha$  and TNF- $\alpha$  by  $\gamma$  $\delta$ T and Th17 cells those lead to a massive inflammation and lung injury. Further, PM<sub>2.5</sub> stimulates the levels of TGF- $\beta$ 1, Smad-dependent TGF- $\beta$  profibrogenic responses including myofibroblast differentiation, excessive collagen synthesis and fibrogenesis [33]. Further, the PM<sub>2.5</sub>-activated profibrogenic pathway is diminished in IL-17A null murine lung tissues compared to wildtype mice indicating IL-17A aggravates PM<sub>2.5</sub>-induced inflammation and lung fibrogenesis [33]. Similarly, exposure to PM<sub>2.5</sub> increases lung injury, decreases lung functions including lung vital capacity and airway resistance through induction of inflammation and oxidative stress in mice and mouse bronchial epithelium cells as evidenced by elevated levels of IL-1 $\alpha$ , IL-16, PI3K/mTOR signaling pathways [34]. Importantly, exposure to low, medium, and high doses of PM<sub>2.5</sub> (3 mg, 8 mg, 13 mg/kg body weight/once per week for 4 weeks) induces worst inflammation and lung injury as shown by increased expression of ACP, CRP, VEGF, and IL-6 in broncho alveolar lavage fluid compared to control rats. Additionally, the protein levels of VEGF, JAK2, Stat3 and matrix protein collagen are significantly elevated in PM<sub>2.5</sub>-treated rat lung tissues compared to controls [35]. These results suggest that PM<sub>2.5</sub>-induced PI3K/mTOR and JAK/Stat3 signaling pathways may contribute to massive lung inflammation and fibrogenesis. Interestingly, exposures of mice to printing room generated PM<sub>2.5</sub> (5 $\mu$ g, 10 $\mu$ g or 15 $\mu$ g/g BW on day 1 and 3) significantly increased malondialdehyde (MDA) activity, increased expression of inflammatory cytokines like IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 and decreased expression of antioxidant SOD on day 4 of exposure. In addition, primary profibrogenic signaling mediator TGF- $\beta$ -induced pERK1-MAPK activity is also increased by PM<sub>2.5</sub> indicating exposure for a significant amount of time to print room-generated PM<sub>2.5</sub> is a major risk factor for increased lung oxidative stress, inflammation, pyroptosis and pulmonary fibrosis [36].

Exposure to PM<sub>2.5</sub> (50 $\mu$ g/mouse/every 3 days/total 6 times) causes increased infiltration of inflammatory cells and lung injury including peri-bronchial fibrosis and airway wall thickening in mice [27]. PM<sub>2.5</sub> (4mg/kg daily for 5days) also significantly increases the levels of CXCL1, IL-6 and IL-18. The levels of Nlrp3/NF $\kappa$ B and Akt signaling are significantly elevated in hearts of PM<sub>2.5</sub> exposed mice. Therefore, Nlrp3/NF $\kappa$ B-induced inflammation may contribute to PM<sub>2.5</sub>-induced cardiac pathologies including fibrogenesis [37]. As HDAC3 plays a key role in regulation of inflammatory genes and control inflammation in response to external stresses, the significance of HDAC3 in PM<sub>2.5</sub>-induced inflammation-related symptoms in mice has been examined [38]. While PM<sub>2.5</sub> inhalation (101.5 $\pm$  2.3  $\mu$ g/ $m^3$ , flow rate: 75L/min for 6h/day/5 time per week) induces the Smad-dependent TGF- $\beta$  signaling in wildtype mice, this profibrogenic signaling is further activated in lungs derived from PM<sub>2.5</sub>-exposed HDAC3 deficient mice [38]. Therefore, specific activation of HDAC3 may be a viable approach to control the extent of PM<sub>2.5</sub>-induced lung inflammation and fibrosis. Exposure to concentrated PM<sub>2.5</sub> (671.87 $\mu$ g/ $m^3$  for 8 or 16 weeks, 6 h/day) also imparts its negative influence on the cardiac structure and function as shown by cardiac hypertrophy, fibrosis, and abnormal cardiac systolic function. PM<sub>2.5</sub> induces inflammation through activation of PI3K/Akt/FOXO1 signaling pathways that contribute to cardiac hypertrophy and fibrogenesis [39]. Furthermore, the offspring

from mice exposed to PM<sub>2.5</sub> during gestation period develop cardiac hypertrophy that is associated with increased levels of acetyltransferase p300, acetylated H3K9 and cardiac transcriptional regulators Gata4 and Mef2c [40]. Therefore, prenatal, or postnatal exposure to environmental pollutant PM<sub>2.5</sub> induces cardiac inflammation, cellular apoptosis, fibrogenesis and abnormal cardiac structure and function.

### 3.4. PM<sub>2.5</sub>-Induced Inflammation, Oxidative Stress, Metabolic Syndrome, and Accelerated Aging

Exposure to PM<sub>2.5</sub> is associated with accelerated aging and metabolic disorders [9,10,41]. Using *Drosophila* as a model for longevity study, Wang and colleagues [42] showed that exposure to concentrated PM<sub>2.5</sub> (80 µg/m<sup>3</sup>) reduces *Drosophila* lifespan in both males and females compared to *Drosophila* exposed to filtered air (PM<sub>2.5</sub>:4 µg/m<sup>3</sup>). Interestingly, males are more sensitive to PM<sub>2.5</sub> than females (50% survival 20-21 days vs 40 days for filtered air exposed flies). It is important to note that PM<sub>2.5</sub> driven *Drosophila* mortality is also associated with increased oxidative stress as evidenced by increased expression of SOD1, Catalase, Thor and Duox as an adaptive responses to PM<sub>2.5</sub>-induced stress; and inflammation as shown by elevated expression of Jak, Jnk and NF-κB in *Drosophila* whole body. Additionally, DCFH oxidation is significantly increased in whole body lysates from concentrated PM<sub>2.5</sub>-exposed flies compared to filtered air exposed flies indicating PM<sub>2.5</sub> induces systemic oxidative stress. Exposure of *Drosophila* for 15 days to concentrated PM<sub>2.5</sub> (6h/day, 5days/week, average concentration of PM<sub>2.5</sub> (17µg and 24 µg.m<sup>3</sup>/24h) also induces abnormal metabolism including deregulated insulin signaling and insulin resistance as evidenced by elevated levels of glucose and trehalose and increased expression of Ilp2 and Ilp5 transcripts in *Drosophila* [42]. Therefore, the results of this *in vivo* study confirmed the negative impact of PM<sub>2.5</sub>-induced inflammation and oxidative stress on organismal metabolism and longevity.

As shortening of telomere length is a bonafide marker of chronological and accelerated aging, the impact of air-pollution exposure on cord blood and placental telomere length in 641 newborns has been investigated [9]. Upon measuring the telomere length in cord blood buffy coat and placental tissues, this study showed that mothers exposed to higher levels of PM<sub>2.5</sub> (5 µg/m<sup>3</sup> increase during entire pregnancy period) gave birth to newborns with significantly shorter telomere length, an indicator of shorter lifespan [9]. Hence, this study further indicates that prenatal exposure to increased levels of air pollutants is associated with accelerated biological aging process. Further, a recent study on the effects of PM<sub>2.5</sub> on *Caenorhabditis elegans* lifespan define that exposure to low dose (94 µg/ml) and high dose (119 µg/ml) of water-soluble component of PM<sub>2.5</sub> (WS-PM<sub>2.5</sub>) significantly shortened the lifespan of *C. elegans*. PM<sub>2.5</sub> imparts adverse effects on healthspan as evidenced by reduced rate of head thrashing and pharyngeal pumping and decreased body length compared to control animal without PM<sub>2.5</sub> exposure under heat stress environment. RNA seq analysis revealed that the adverse effects of PM<sub>2.5</sub> on nematode lifespan and healthspan are associated with deregulation in insulin/IGF-1 signaling and fat metabolism [10]. Collectively, the results of these *in vivo* studies clearly indicate the deleterious effects of PM<sub>2.5</sub> exposure on organismal lifespan and healthspan. Further research is needed to determine the molecular basis underlying the negative effects of PM<sub>2.5</sub> on mammalian lifespan and healthspan using suitable mammalian models.

It is evident from the above-discussed studies that for each investigation, different experimental milieu in terms of sources, concentration, heterogeneity in the composition of particulate matter, time of collection, period of exposure to PM<sub>2.5</sub>, cell lines and animal models are used. However, despite the experimental heterogeneity, the results of all the studies provide clear and convincing evidence that PM<sub>2.5</sub> induces massive inflammation and oxidative stresses, the root causes of all air pollutant-induced multi-organ pathologies and accelerated biological aging process.

## 4. Efficacies of Natural and Synthetic Compounds in Alleviation of PM<sub>2.5</sub>-Induced Inflammation, Oxidative stress, and Diseases

In this section, I discuss the recent findings on the efficacies of different synthetic and natural compounds in amelioration of PM<sub>2.5</sub>-induced sustained oxidative stress, inflammation and associated pathologies using animal and cellular models.

### 4.1. Lessons from Studies Using Animal Models and Synthetic Compounds

The potential of different synthetic molecules to alleviate PM<sub>2.5</sub>-induced inflammation, oxidative stress, and associated pathologies have been evaluated in preclinical settings. A wealth of research demonstrates that an imbalance in the level of plasminogen activator inhibitor-1 (PAI-1), the most potent inhibitor of serine proteases uPA/t-PA, is associated with a wide variety of diseases including cardiovascular, pulmonary, metabolism and accelerated aging, and upregulated by the exposure to PM<sub>2.5</sub> [43-49]. Recently, we evaluated the efficacy of a drug-like small molecule inhibitor *TM5614* targeting PAI-1 in amelioration of PM<sub>2.5</sub>-induced pulmonary and cardiac pathologies. A short-term exposure (24 h) of mice to PM<sub>2.5</sub> (50 µg/mouse) increases the levels of circulatory PAI-1, inflammatory cytokine IL-6 and thrombin, a coagulation factor involved in vascular thrombosis. Interestingly, PM<sub>2.5</sub> did not increase the levels of circulatory PAI-1, thrombin, and IL-6 in mice pretreated with PAI-1 inhibitor *TM5614* (10mg/kg/day). Importantly, PAI-1 specific inhibitor *TM5614* diminishes short-term (72h) PM<sub>2.5</sub> exposure (200 µg/mouse/once)-induced inflammatory markers Mac3, pStat3 and Vcam1, and apoptotic marker cleaved caspase 3 in lung and cardiac tissues [28]. Analysis of RNA seq data reveals while PM<sub>2.5</sub> (200 µg/mouse once in 72 h) induces the inflammatory factors including Nlrp3, IL-1 $\alpha$ , NF $\kappa$ B2, TNF $\alpha$ 11a, TNF $\alpha$ 12a, pretreatment of mice with *TM5614* (10 mg/kg/day) prevents induction of these inflammation mediators (Ghosh et al. unpublished data). After long-term exposure to PM<sub>2.5</sub> (100 µg/mouse/week for 4 weeks), mice develop lung and heart vascular thrombosis. Most importantly, pretreatment with *TM5614* significantly decreases PM<sub>2.5</sub>-induced vascular thrombosis in lungs and hearts [28]. Therefore, air pollutant PM<sub>2.5</sub>-induced inflammation, apoptosis and vascular thrombosis can be controlled by promising drug-like small molecule *TM5614* targeting PAI-1, a pro-thrombotic and pro-aging factor. Future preclinical study using large animal cohort is required to proceed for clinical trials of this drug for treatment of air-pollutant-induced pathologies.

Exposure to PM<sub>2.5</sub> (120 µg/ml for 14 days) causes massive lung inflammation and lung injury like alveolar structure disruption in mice. Importantly, PM<sub>2.5</sub> augments the levels of inflammatory cytokines like TNF- $\alpha$ , IL-6, and IL-1 $\alpha$ , inflammasome Nlrp3 and apoptotic caspase pathway both in mouse and 16HBE cell (20 µg/ml/24h) models. Significantly, PM<sub>2.5</sub> exposure-induced lung inflammation and pyroptosis are blocked by the pretreatment of mice with Nlrp3-specific inhibitor *MCC950* (2.5 mg/kg) suggesting targeting Nlrp3 with small molecule inhibitor is a practical approach to control PM<sub>2.5</sub>-induced persistent inflammation and pyroptosis-driven lung pathologies [50]. Furthermore, exposure of 16HBE cells to PM<sub>2.5</sub> (10-40 µg/ml) causes elevated IL-1 $\alpha$  expression, increased small GTPase Rac1 and increased inflammation. However, pretreatment of 16HBE for 30 min with Rac1 inhibitor *NSC23766* suppresses PM<sub>2.5</sub>-induced IL-1 $\alpha$  secretion. This study also showed that pharmacological inhibition of Rac1 with *NSC23766* (1mg/kg for 9 days; 30 min pretreatment before PM<sub>2.5</sub> exposure) blocks PM<sub>2.5</sub> (100 µg/every 3<sup>rd</sup> day for 9 days)-induced increased IL-1 $\alpha$  secretion, infiltration of neutrophils and macrophages in murine lungs [51]. Therefore, Rac1 may be a druggable target for therapy of PM<sub>2.5</sub>-induced increased inflammation and associated lung diseases.

#### 4.2. Lessons from Studies Using Animal Models and Natural Compounds

Here, I discuss the efficacies of several natural compounds in alleviation of PM<sub>2.5</sub>-induced pathologies ignited by PM<sub>2.5</sub>-induced inflammation and oxidative stresses. As *Salvianolic acid B* (*SalB*) is a known strong anti-oxidative and anti-inflammatory natural agent [52], a recent study evaluated the efficacy of *SalB* (0.3 mg/kg, 0.9 mg/kg and 1.8mg/kg) inhalation on PM<sub>2.5</sub> (10 µg daily for 5 days)-induced inflammation and oxidative stress in mice [53]. Treatment with *SalB* significantly reduces PM<sub>2.5</sub>-induced infiltration of neutrophil and macrophage, expression levels of IL-1 $\alpha$ , TNF- $\alpha$ , KC, TGF- $\beta$  $\alpha$ , TLR4, MyD88, TRAP6 and Nlrp3 in a dose-dependent manner and thus alleviates inflammation in the lung tissues. Importantly, treatment of PM<sub>2.5</sub>-exposed mice with *SalB* rescued PM<sub>2.5</sub>-induced suppression of antioxidant genes SOD, CAT, GSH and GSH-Px in mouse lungs [53]. These results clearly suggest *SalB* is highly effective in alleviation of PM<sub>2.5</sub>-induced inflammation, oxidative stress and thus abnormal lung structure and function.

The therapeutic efficacy of steroidal alkaloid *Sipeimine*, an anti-inflammatory and anti-asthmatic agent, has been evaluated in amelioration of PM<sub>2.5</sub>-induced lung inflammation and injury [54]. Pretreatment of mice with *Sipeimine* (30 mg/kg/day/for 3 days) blocks PM<sub>2.5</sub> (7.5 mg/kg/day for 2days)-induced lung inflammation, pulmonary edema, and injury through suppression of inflammatory cytokines TNF- $\alpha$ , IL-1 $\alpha$  and oxidative stress through reversal of PM<sub>2.5</sub>-induced increased MDA and decreased GSH. Importantly, *Sipeimine* blocks PM<sub>2.5</sub>-induced inhibition of Nrf2, the primary regulator

of antioxidant genes, and thus diminishes oxidative stress [54]. These results implicate the therapeutic potential of *Sipeimine* for the treatment of PM<sub>2.5</sub>-induced lung pathologies through inhibition of inflammation and oxidative stress. Additionally, pretreatment of Sprague-Dawley rats with *Sipeimine* (15 mg/kg-30 mg/kg) for 3 days cause significantly decreases PM<sub>2.5</sub> (7.5mg/kg)-induced lung injury-related damage that is accompanied by reduced levels of inflammatory IL-1 $\alpha$ , IL-18, TNF- $\alpha$ , Nlrp3 and apoptotic caspase. Thus, *Sipeimine* effectively ameliorates PM<sub>2.5</sub>-induced inflammation, pyroptosis and lung injury. This has been further supported by the observation that the beneficial effect of *Sipeimine* is blocked by pretreatment with Nlrp3 activator nigericin [55]. Similarly, *Astragaloside IV* (AS-IV), a plant product from *Astragalus Membranaceus* with anti-oxidative and anti-inflammatory properties, is highly effective in amelioration of PM<sub>2.5</sub>-induced massive lung pathologies in a rat model [56,57]. Pretreatment of rats with AS-IV (50-100 mg/kg/day/for 3 days) improved PM<sub>2.5</sub> (7.5 mg/kg/day)-induced lung injury as shown by the decreased inflammatory signaling molecules IL-6, TNF- $\alpha$ , CRP, TLR4 and NF $\kappa$ B pathways and oxidative stress in lungs [56,57]. Further, AS-IV inhibits PM<sub>2.5</sub>-induced PI3K/mTOR pathway and NF- $\kappa$ B translocation in NR8383 rat macrophages. In addition, AS-IV blocks PM<sub>2.5</sub>-induced suppression of antioxidant genes SOD and CAT [57]. Importantly, pretreatment of mice with AS-IV (50-100 mg/kg) also reduces PM<sub>2.5</sub> (7.5 mg/kg/twice, 0, 24h followed by harvest at 36h)-induced inflammation, oxidative stress and pyroptosis through Nlrp3 pathway because pretreatment with Nlrp3 activator nigericin diminishes beneficial effect of AS-IV on PM<sub>2.5</sub>-induced lung pathologies [58]. Therefore, the bioactive herbal substance AS-IV has therapeutic potential in amelioration of PM<sub>2.5</sub>-induced inflammation and oxidative stress-driven lung pathologies. Thus, AS-IV may be a future potential drug to control PM<sub>2.5</sub>-induced lung injury and Nlrp3 is a potent druggable target for therapy.

The efficacy of *Tussilagone* (TLS), a natural compound derived from flower bud, in amelioration of PM<sub>2.5</sub>-induced lung pathologies has been evaluated [59]. Treatment of mice with TLS (20mg/kg/every 3 days) blunts PM<sub>2.5</sub> (20mg/kg/4h inhalation/day for 6 days)-induced ROS production or oxidative stress, lung inflammation as shown by reduced levels of IL-1 $\alpha$ , IL-6, IL-12 and TNF- $\alpha$  and injury through downregulation of PM<sub>2.5</sub>-induced HIF-1 $\alpha$  and NF $\kappa$ B signaling. In addition, pretreatment of human lung epithelial cells (A549) with TLS (25  $\mu$ g/ml) reduces PM<sub>2.5</sub> (30  $\mu$ g, 100  $\mu$ g, 300  $\mu$ g/ml for 4 days)-induced apoptosis markers like cleaved caspase 3 and LDH activity, and inflammatory cytokines IL-1 $\alpha$ , IL-6, and TNF- $\alpha$  [59]. Collectively, these results indicate the therapeutic potential of TLS for the treatment of air pollution-induced lung inflammation and oxidative stress. The therapeutic efficacy of *Deng-Shi-Qing-Mai-Tang* (DSQMT), a Chinese herbal formula, on PM<sub>2.5</sub>-induced lung injury has been assessed [60]. Treatment with DSQMT (3 ml of 0.72, 1.45, 2.90 g/ml) significantly decreases the inflammatory cytokines IL-1 $\alpha$ , IL-6, and TNF- $\alpha$  and pathologies like damaged lung tissues and higher lung permeability index in rats exposed to PM<sub>2.5</sub> (50  $\mu$ g/rat/week for 8 weeks). Additionally, DSQMT (20% of medicated serum 1.45g/ml) decreases the PM<sub>2.5</sub> (0.5mg/ml)-induced increased expression of many factors involved in inflammation including IL-1 $\alpha$ , IL-6 and TNF- $\alpha$  in rat alveolar macrophages, NR8383 [60]. Thus, this study implicated DSQMT as a potential natural compound to control air pollution-induced lung injury through modulation of PM<sub>2.5</sub>-induced inflammatory responses. As *Schisandrae Fructus* fruit is known to possesses the anti-inflammatory and antioxidant activities, the therapeutic efficacy of *Schisandrae Fructus ethanol extract* (SF) (200  $\mu$ g and 400  $\mu$ g/ml pretreated for 1h) on PM<sub>2.5</sub> (50  $\mu$ g/ml for 24h)-induced inflammatory and oxidative stress developed in RAW264.7 macrophages and post fertilized (day3) zebrafish larvae has been evaluated [61]. Significantly, SF reduces the expression of PM<sub>2.5</sub>-induced inflammatory cytokines IL-6 and IL-1 $\alpha$ , NO and COX2 through disruption of nuclear translocation of NF $\kappa$ B from cytoplasm to nucleus and impaired NF $\kappa$ B signaling. Pretreatment with SF also blocks PM<sub>2.5</sub>-induced ROS activity in macrophages and zebrafish larvae as shown by ROS fluorescence intensity [61]. Therefore, SF with anti-inflammatory as well as antioxidative properties is an excellent choice for the treatment of oxidative stress- and inflammation-induced tissue damages. Future *in vivo* studies are needed to explore the therapeutic efficacy of SF in amelioration of PM<sub>2.5</sub>-induced massive inflammation and oxidative stress in mammalian models.

*Bergapten* (5-methoxysporalen), a bergamont essential oil, possesses antioxidant and anti-inflammatory properties. While exposure to PM<sub>2.5</sub> (100  $\mu$ g/mouse for 30 days) aggravates OVA-induced combined allergic rhinitis and asthma syndrome (CARAS) with massive lung inflammation and lung injury in mice, treatment of mice with *Bergapten* (3,10,30 mg/kg) induces OVA-specific



IgG2A and decreases the level of IgE and IgG1 in serum. Most importantly, *Bergapten* reduces the inflammation in nasal mucosa and lungs through induction of Th1 cytokine IL-12, IFN- $\gamma$  and reduction of Th2 cytokines IL-4, IL-5, and IL-13 [62]. These results indicate that *Bergapten* is a potential natural therapeutic agent to treat CARAS and PM<sub>2.5</sub>-induced worst lung pathologies. Similarly, the efficacy of *Rosavidin*, a phenylpropanoid compound having multiple biological activities extracted from the *Rhodiola crenulata* plant, in amelioration of PM<sub>2.5</sub>-induced lung pathology has been examined in a rat model. Pretreatment of rats with *Rosavidin* (50-100 mg/kg/day for 3 days) diminishes PM<sub>2.5</sub> (7.5mg/kg twice in 36h at 0h and 24h)-induced inflammation and ameliorates lung pathologies in rats through inhibition of inflammatory and apoptotic regulators including IL-1 $\alpha$ , Nlrp3 inflammasome, and caspase. This study further demonstrated that Nlrp3 specific activator nigerin blunts *Rosavidin*-mediated amelioration of PM<sub>2.5</sub>-induced lung pathologies [63]. Therefore, *Rosavidin* has potential to be a remedy to controlling PM<sub>2.5</sub>-induced inflammation and pyroptosis-driven lung pathologies. It is well documented that exposure to PM<sub>2.5</sub> causes worst lung pathologies in COPD patients [64,65]. *Buwei Yishen formula* (ECC-BYF), a Chinese herbal medicinal formula, efficiently improves COPD in a rat model that was developed by repeated cigarette smoke inhalation (2 times daily, 30 min each time for 8 weeks and intranasal instillation of pneumonia bacteria once for every 5 days). Whole body exposure of COPD rats to PM<sub>2.5</sub> for another 8 weeks (average daily conc. of PM<sub>2.5</sub> 739.97 $\mu$ g/m<sup>3</sup>; 4h/day for 8 weeks) leads to excessive lung inflammation, lung tissue remodeling and decreased lung function in this rat model of COPD. However, PM<sub>2.5</sub> failed to induce inflammation, oxidative stress, pyroptosis and excessive collagen deposition in the lungs of ECC-BYF-treated COPD rat model [66]. These results clearly indicate the therapeutic efficacy of ECC-BYF for the treatment of PM<sub>2.5</sub>-induced worst lung inflammation, pyroptosis and lung injury in COPD in a preclinical setting.

As *Juglanin* is a plant product with anti-inflammatory and anti-oxidative properties, the therapeutic efficacy of *Juglanin* on PM<sub>2.5</sub>-induced inflammation, oxidative stress, and liver injury has been assessed [67]. Interestingly, *Juglanin* (40mg/kg/day, via gavage 6h prior to PM<sub>2.5</sub> exposure) reduces PM<sub>2.5</sub> (151.1  $\pm$  2.5  $\mu$ g/m<sup>3</sup>, 6 h/day, 5 times/week for 24 weeks)-induced liver injury through activation of antioxidant gene regulator Nrf2, and suppressor of IKK $\epsilon$  (SIKE), a known negative regulator of inflammatory signaling. It is important to note that Nrf2 and SIKE KO mice are more susceptible to PM<sub>2.5</sub>-induced oxidative stress/ROS generation as shown by higher level of MDA, lower level of SOD, and increased inflammation as shown by higher IL-1 $\alpha$ , IL-6, TNF- $\alpha$ , and liver injury as shown by higher ALT and AST compared to wildtype mice. These *in vivo* observations on the beneficial effects of *Juglanin* on PM<sub>2.5</sub>-induced liver injury have also been replicated *in vitro* using human liver cell line LO2 [67]. Together, this study suggests the significant involvement of Nrf2 and SIKE pathways in PM<sub>2.5</sub>-induced liver injury and most importantly, *Juglanin* is a potential therapeutic agent to controlling PM<sub>2.5</sub>-induced inflammation, oxidative stress, and liver pathologies. A recent study also showed that Nrf2 protects PM<sub>2.5</sub> (20mg/kg)-induced lung injury through its regulation of iron-dependent cellular death or ferroptosis. This is supported by the observation that ferroptosis and lung injury in response to PM<sub>2.5</sub> are more severe in Nrf2-deficient lung tissue and cellular model [68]. Similarly, *Tectoridin* (50-100 mg/kg), a bioactive molecule, also ameliorates PM<sub>2.5</sub> (20mg/kg for 7 days)-induced lung injury as revealed by decreased morphological damage, necrosis, edema and inflammation with decreased IL-6 and TNF- $\alpha$  through stimulation of antioxidant gene regulator Nrf2 and antioxidant genes like GSH and GPX4. Similarly, pretreatment of BEAS-2B cells with *Tectoridin* (25, 50 and 100  $\mu$ M for 1 h) reduces PM<sub>2.5</sub> (400 $\mu$ g/ml for 24h)-induced ROS generation through activation of Nrf2, GSH and inhibition of PM<sub>2.5</sub>-induced inflammatory MDA [68]. These results suggest that *Tectoridin* has potential to controlling PM<sub>2.5</sub>-induced oxidative stress, ferroptosis, and lung pathologies. It is known that exercise-induced myokine, *Irisin*, a polypeptide derived from muscle and adipose tissues, is a potent anti-inflammatory agent that diminishes metabolic syndrome [69]. Interestingly, pretreatment of mice with recombinant *Irisin* (250  $\mu$ g/kg) significantly diminishes the PM<sub>2.5</sub> (8mg/kg for 24h)-induced increased level of inflammatory cytokines IL-1 $\alpha$ , IL-18, TNF- $\alpha$  and mediators of inflammation including NF- $\kappa$ B, and Nlrp3 inflammasome [70]. Therefore, *Irisin* is an effective myokine in amelioration of PM<sub>2.5</sub>-induced lung pathologies through suppression of inflammatory pathways.

Collectively, the results from all these studies in this section strongly suggest that irrespective of the unique characteristics of each natural compound and doses used, all the tested compounds are

efficacious in diminishing PM<sub>2.5</sub>-induced pathologies through suppression of massive inflammation and oxidative stress. However, further long-term *in vivo*, and *in vitro* studies are essential to understand in-depth the underlying molecular mechanisms by which these natural compounds govern the factors/mediators involved in inflammation and oxidative stress.

#### 4.3. Lessons from Studies Using Cellular Models and Synthetic Compounds

In this section, I discuss the major findings on the efficacies of several synthetic and natural compounds in amelioration of PM<sub>2.5</sub>-induced cellular abnormalities including activation of oxidative stress and inflammatory pathways using cellular models.

Fine particulate matter (PM<sub>2.5</sub>)-induced detrimental effects on endothelial cells, the first cellular barrier of the cardiovascular system, have been well studied. To investigate the contribution of oxidative stress and inflammation on PM<sub>2.5</sub>-induced endothelial injury, the effect of PM<sub>2.5</sub> on EA.hy926 endothelial cells was examined [71]. PM<sub>2.5</sub> exposure (50 µg/ml for 24h) induces NOX1/4, superoxide, H<sub>2</sub>O<sub>2</sub>, ET1 and decreases NO pathway. Furthermore, PM<sub>2.5</sub> causes an imbalance in the ratio of t-PA to PAI-1 due to significantly increased expression of PAI-1 and decreased expression of t-PA. Exposure to PM<sub>2.5</sub> also augments the expression levels of inflammatory cytokines including IL-1 $\alpha$  and IL-18 in this cell line, indicating PM<sub>2.5</sub> exposure contributes to endothelial dysfunction. Importantly, pretreatment of EA.hy926 cells with NOX1/4 inhibitor (GSK 13783) (5 $\mu$ M) diminishes PM<sub>2.5</sub>-induced oxidative stress and inflammation and thus ameliorates PM<sub>2.5</sub>-induced endothelial dysfunction [71]. Hence, NOX1/4 may be a druggable target to reduce air pollutant PM<sub>2.5</sub>-induced endothelial dysfunction and associated cardiovascular diseases. The pharmacological effect of *Ropivacaine*, a widely used local anesthetic, on PM<sub>2.5</sub>-induced acute lung injury has been explored in cultured lung cells [72]. Exposure to PM<sub>2.5</sub> (100µg/ml) induces the inflammatory and oxidative stress in lung cells BEAS-2B as shown by increased expression of inflammatory cytokines IL-6, IL-8, IL-1 $\alpha$ , TNF- $\alpha$  and oxidative stress-related MDA, and decreased expression of GSH. However, pretreatment of BEAS-2B cells with *Ropivacaine* (1 µM, 10 µM, 100 µM) reduces PM<sub>2.5</sub>-induced inflammatory pathway, oxidative stress, and cell death through downregulation of inflammasome Nlrp3 and apoptotic caspase pathways [72], indicating *Ropivacaine* has potential to reduce PM<sub>2.5</sub>-induced inflammation, oxidative stress, and thus may be effective in diminishing lung injury-associated pathologies. Similarly, pretreatment of human bronchial epithelial cells (16HBE) with Caspase inhibitors Z-VAD-FMK and VX-765 block wood smoke-derived PM<sub>2.5</sub> (5, 10, 20 µg/ml)-induced inflammation and pyroptosis of 16HBE cells as evidenced by decreased levels of LDH activity, caspase, inflammatory cytokines IL-1 $\alpha$  and IL-18, the downstream targets of Nlrp3 [19]. These results show the potential of caspase inhibitors to block wildfire/wood smoke-induced massive inflammation and pyroptosis.

As *Vitamin D<sub>3</sub>* possesses anti-inflammatory activity, the therapeutic potential of *VitD<sub>3</sub>* in PM<sub>2.5</sub>-induced inflammation has been assessed in human bronchial epithelial cells (16HBE) [73]. PM<sub>2.5</sub> (200µg/ml for 48h)-treated 16HBE cells produce elevated levels of ROS and MDA, and the secretion of inflammatory mediators IL-6, IL-18, NF $\kappa$ B and Nlrp3 inflammasome. However, pretreatment of 16HBE with *VitD<sub>3</sub>* (1nM) for 24h decreases the PM<sub>2.5</sub>-induced ROS generation, and expression of MDA, IL-6, IL-8, NF $\kappa$ B and Nlrp3, indicating *VitD<sub>3</sub>* is effective in inhibition of PM<sub>2.5</sub>-induced inflammatory and oxidative stress responses [73]. Similarly, pretreatment of rat neonatal cardiomyocytes with *VitD<sub>3</sub>* (10<sup>-8</sup> mol/L) significantly reduce the cooking oil fumes-derived PM<sub>2.5</sub> (50 µg/ml)-induced ROS production, inflammation and pyroptosis through suppression of inflammatory signaling pathways JAK/Stat1 and NF $\kappa$ B. Further, *VitD<sub>3</sub>* also prevents PM<sub>2.5</sub>-induced inhibition of antioxidant SOD and GSH in cardiomyocytes [74]. Collectively, these results indicate that *VitD<sub>3</sub>* is cardioprotective from PM<sub>2.5</sub>-induced inflammation, oxidative stress, and associated pathologies. Another study [75] showed that while the expression levels of inflammatory TLR4, NF $\kappa$ B and COX2 are significantly increased in PM<sub>2.5</sub> (250 µg/ml for 24-72 h)-treated RAW254.7 macrophages, pretreatment with TLR4-inhibitor TAK242 (5-20 µM) significantly inhibits PM<sub>2.5</sub>-induced pro-inflammatory signaling molecules IL-6, MCP1 and TNF- $\alpha$  [75]. Therefore, TLR4-specific inhibitor has potential to controlling PM<sub>2.5</sub>-induced inflammation. Similarly, the levels of inflammatory markers IL-1 $\alpha$ , COX2 and oxidative stress marker Hmox1 are also significantly elevated in PM<sub>2.5</sub>-exposed (30 µg/ml for 3h) mouse macrophages. While PM<sub>2.5</sub>-induced inflammatory responses are decreased in macrophages either by pretreatment with endotoxin neutralizer *polymyxin B* (0.5mg/ml) or NF-kB

inhibitor Bay 11-7085 (10  $\mu$ M), the oxidative stress responses are decreased by antioxidant *n*-acetyl cysteine (NAC) (10mM) [76]. Collectively, the results of these *in vitro* studies provide clear evidence that PM<sub>2.5</sub>-induced inflammation and oxidative stress pathways can be effectively blocked by different synthetic compounds.

#### 4.4. Lessons from Studies Using Cellular Models and Natural Compounds

It is known that exposure to PM<sub>2.5</sub> not only affects lungs and cardiovascular system but also affects brain and cognitive functions. Air pollutant PM<sub>2.5</sub> can reach to the brain and contributes to accelerated neurological syndromes including Alzheimer's disease [77,78]. As carotenoid, *Astaxanthin* is a known anti-inflammatory and neuroprotective agent, the efficacy of *Astaxanthin* on PM<sub>2.5</sub>-induced inflammation and neurotoxicity has been evaluated and demonstrated that PM<sub>2.5</sub> stimulates the levels of ROS/oxidative stress, inflammatory mediators IL-1 $\alpha$ , IL-6, TNF- $\alpha$ , TLR2/4, and COX2 and stress-induced protein HO-1 in BV-2 microglial cells. Most importantly, PM<sub>2.5</sub> (50  $\mu$ g/ml/24h) failed to induce the inflammatory markers in rat glial cells pretreated with *Astaxanthin* (1, 10  $\mu$ g/ml) for 4 h. *Astaxanthin* also prevents PM<sub>2.5</sub>-induced inhibition of IL-10 and Arg-1. Hence, *Astaxanthin* is effective in prevention of PM<sub>2.5</sub>-induced inflammation, oxidative stress and associated neurological disorders [79]. The plant product *Ophiopogonin D* is also an anti-inflammatory agent. Pretreatment of mouse lung epithelial cells MLE-12 with *Ophiopogonin D* (10-80  $\mu$ M) for 1h inhibits PM<sub>2.5</sub> (15  $\mu$ g/cm<sup>2</sup> for 24h)-induced inflammation as shown by the decreased levels of IL-1 $\alpha$ , IL-6, IL-8, and TNF- $\alpha$ . The *Ophiopogonin D* exerts its anti-inflammatory effect through downregulation of NF $\kappa$ B signaling and activation of AMPK activity as pretreatment of cells with AMPK inhibitor (Compound C, 10  $\mu$ M) blocks anti-inflammatory activity of *Ophiopogonin D* [80]. As the dihydrophenanthrene *Coelonin*, derived from the flowering plant *Bletilla striata*, is a known anti-inflammatory agent [81,82], its therapeutic efficacy in amelioration of PM<sub>2.5</sub>-induced inflammation has been evaluated [83]. Pretreatment with *Coelonin* (1.25, 2.5 or 5  $\mu$ g/ml for 2h) ameliorates PM<sub>2.5</sub> (200 $\mu$ /ml for 18h)-induced inflammation, oxidative stress and pyroptosis of RAW264 and 1774A.1 macrophages through suppression of Nlrp3 inflammasome, IL-6, TNF- $\alpha$ , TLR4, COX2, and NF $\kappa$ B signaling [83]. These results suggest that different natural compounds are effective in diminishing PM<sub>2.5</sub>-induced massive inflammation, oxidative stress, and pyroptosis.

Therefore, the results of all these cell biology studies suggest that pharmacological modulation of inflammatory mediators or oxidative stress regulators are ideal therapeutic approaches to controlling air-pollutant PM<sub>2.5</sub>-induced disease development. However, more in-depth preclinical studies using proper models are necessary to reproduce the efficacies of these natural and synthetic compounds before proceeding for clinical trials.

## 5. Concluding Remarks

Air pollution is one of the major risk factors to human health and shortening of healthspan worldwide. In search of remedies for the air pollution driven stress-induced health risk, many investigations have been undertaken worldwide as discussed in this article. A careful analysis of all these preclinical studies on air pollutant PM<sub>2.5</sub> and its impact on organismal health unequivocally proved the pivotal contribution of PM<sub>2.5</sub>-induced inflammation and oxidative stress in initiation and progression of a wide variety of pathologies and accelerated aging process. Hence, the development of drug-like small molecules targeting PM<sub>2.5</sub>-deregulated pathogenic factors will be a promising approach for amelioration of PM<sub>2.5</sub>-induced oxidative stress, inflammation, and associated pathologies. Meta-analysis of related published data set on air-pollution deregulated molecules, cellular and biological processes may be helpful to identify unique and common pathogenic factor(s). Based on the observations made by different independent investigations under different experimental milieus as has been discussed earlier in this article, it is noticeable that while the expression level of antioxidant gene regulator Nrf2 is decreased, the levels of inflammasome Nlrp3 and pro-aging factor PAI-1 are significantly elevated in response to air pollutant PM<sub>2.5</sub> exposures. Therefore, development of natural or synthetic drugs either, as an activator targeting Nrf2 or repressor/inhibitor targeting Nlrp3 or PAI-1 will be a feasible approach to abate air pollution-induced initiation of multiorgan pathologies [see Graphical Abstract]. Further, it is crucial to evaluate the efficacies of the above-discussed natural and synthetic compounds in diminishing PM<sub>2.5</sub>-induced

oxidative stress, inflammation, and cell death by large cohort studies in an unbiased preclinical setting. To rule out the possible harmful effects, it is also crucial to determine the toxicity of each synthetic as well as natural compound after long-term use in control and PM<sub>2.5</sub>-exposed animal models. An identification of the most efficacious and non-toxic safe compound for the clinical trial and its success will save billions of people worldwide from air pollution-induced devastating diseases, and thus will increase the healthspan.

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