Varied dose exposures to ultrafine particles in the motorcycle smoke cause kidney cell damages in male mice

Arinto Y.P. Wardoyo*, Unggul P. Juswono, Johan A.E. Noor

Department of Physics, Brawijaya University, Malang Indonesia Jl. Veteran, Malang, East Java, 65145, Indonesia

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ABSTRACT

Ultrafine particles (UFPs) are one of motorcycle exhaust emissions which can penetrate the lung alveoli and deposit in the kidney. This study was aimed to investigate mice kidney cell physical damage (deformation) due to motorcycle exhaust emission exposures. The motorcycle exhaust emissions were sucked from the muffler with the rate of 33 cm$^3$/s and passed through an ultrafine particle filter system before introduced into the mice exposure chamber. The dose concentration of the exhaust emissions was varied by setting the injected time of the 20s, 40s, 60s, 80s, and 100s. The mice were exposed to the smoke in the chamber for 100 s twice a day. The impact of the ultrafine particles on the kidney was observed by identifying the histological image of the kidney cell deformation using a microscope. The exposure was conducted for 10 days. The kidney observations were carried out on day 11. The results showed that there was a significant linear correlation between the total concentration of ultrafine particles deposited in the kidneys and the physical damage percentages. The increased concentrations of ultrafine particles caused larger cell deformation to the kidneys.

1. Introduction

The number of motor vehicles in the world has been increased from year to year. In Ho Chi Minh City, Vietnam, the road was dominated by light gasoline vehicles, such as motorcycles (92%), cars (3.46%), and light trucks (2.8%) [1]. Motor vehicle emissions have become significant contributors to air pollution that need considerable attention due to their influences on air quality and human’s health [2]. As the implication of the increased motor vehicles, a number of their emissions in ambient air will be increased too. The emissions have been identified as NO$_2$, particulate matters, polycyclic aromatic hydrocarbons, black elemental carbons, hopanee, stearanes [3], nonvolatile particles [4], SO$_2$, and CH$_4$ [1]. The elements of volatile organic compounds, such as isopentane, toluene, and o-xylene can be found in the motorcycle exhaust emission during real-world driving [5].

Previous studies have examined the health effects of ambient air ultrafine particles. They have found the association between ambient ultrafine particles and relevant organ health disorders due to their composition and toxicity. They might be able to penetrate alveoli and deposit to the alveolar surface area [6]. They follow the bloodstream, and harm lung [7], brains [8], and erythrocytes [9]. The ultrafine particle impacts on health are still needed to be investigated deeply. Because of their size, ultrafine particles induce oxidative stress and steatosis in hepatocytes [10], and cause postnatal immunological dysfunction [11]. However, there are limited studies of the impacts of ultrafine particles emitted by motor vehicles, and no available information of the effect of the ultrafine particles on kidney damage. This study was aimed to investigate the effects of the motorcycle exhaust emissions exposure to male mice to get the correlation between the ultrafine particles dose concentration and the kidney cell physical damage (cell deformation).

2. Materials and methods

2.1. Vehicle sample

Five automatic transmission motorcycles (engine capacities: 125 cm$^3$) were selected based on the popularity at Malang (Indonesia). They were classified into M1 (model year: 2009), M2 (model year: 2011), M3 (model year: 2012), M4 (model year: 2013), and M5 (model year: 2015).

2.2. Experimental animals and treatments

All experimental animal treatments were in accordance with the international ethics guidelines and approved by the Animal Care And Use Committee of Brawijaya University Malang, Indonesia (Ethical Clearance No:541-KEP-UB). There were 31 male mice (mean body
weight: 22.7 ± 0.1 g) with the age of 10–12 weeks old. The mice were treated humanely and with regarding the suffering alleviation by providing food and water ad libitum. They were acclimated to the experiment environment (exposure chamber 20 × 20 × 30 cm³) for 3 days [9]. The temperature of housing facility was maintained at 24–27 °C with a relative humidity of 75–78% and a 12 h light/dark cycle. After the acclimatization process, they were randomly divided into the control group (n = 6) and the treatment groups (M1; M2; M3; M4; M5; n = 5/group) and housed separately in the acrylic cages.

2.3. Exposure dose measurements

The motorcycle samples were operated for 60 s for the engine warming up. The muffler was connected to an ultrafine particle filtering system containing a cylindrical tube (diameter: 2.54 cm), an N95 filter paper (3 M 8210 Particulate Respirator), and a sucking pump. The exhaust emissions from the motorcycles were filtered using the filtering system. The system lets the particles with the diameter less than 0.1 μm (ultrafine particles). The exposures of motorcycle exhaust emission doses were adjusted by setting of the sucking time for 20, 40, 60, 80, and 100 s to get the varied concentration of C1, C2, C3, C4, and C5. After passing through the filtering system, the exhaust emissions were introduced into the exposure chamber with the rate of 33 cm³/s. After sucking the exhaust emissions, the pump was turned off and disconnected from the exposure chamber using a valve. The ultrafine particle concentration was measured using a P-Trak UPC (TSI, model 8525) (Fig. 1) [9].

The total concentration of ultrafine particles (Ct) was calculated by summing the measured concentrations (Ct)

\[ C_t = \sum_{i=1}^{\text{all}} C_t \] (1)

2.4. Mice exposures

The mice were exposed to the ultrafine particles with the concentration of C1, C2, C3, C4, and C5 for 100 s. Every group of mice was exposed to the filtered exhaust emission with the concentration of C1, C2, C3, C4, and C5 for 100 s for the ultrafine particle inhalation regarding their tidal volumes and respiratory rates [13]. Our previous research gave us information that the mice collapsed for the exposure of more than 100 s. The control mice (n = 6) were not exposed to the filtered exhaust emissions. After the exposures, they have been released to their origin cages. All mice were sacrificed in the day 11 [9].

2.5. Preparation and histopathologic examination

The mice from each group (n = 1/group) were sacrificed by a cervical dislocation [14]. Their kidneys were fixed in a 10% buffered formalin [15] for a histopathologic examination as long as seven days. After that, they were dehydrated through upgraded ethanol series, trimmed, and processed to paraffinization. The sections were cut using microtome (4 μm thickness) and stained routinely with hematoxylin and eosin. The observations were performed using a computer microscope (Olympus, BX-31). The histopathological examination was performed by scoring the kidney cell deformation level (physical parameter evaluation) of five random fields taken from the 400× magnification of each section [16,17]. In each field, the number of normal and abnormal tubular epithelial cells were counted, while the level of Bowman’s space was measured (Fig. 2) [18]. Normal tubular epithelial cells had no edema and vacuolation [16]. A normal glomerulus indicated a normal organize appearance of glomerulus with a regular Bowman’s space. The abnormal glomerulus indicated a loss of integrity, atrophy, and increasing of Bowman’s space. The deformation level of the glomerulus was focused on the capsular space and was classified into 20%, 40%, 60%, 80%, and 100% wider than the control. Eq. (2) was used to convey the physical damage percentage of the kidney cells.

\[ \text{Physical Damage} (\%) = \left( \frac{\text{Sigma abnormal tubules}}{\text{Sigma tubules}} \times 100 \right) + \% \text{ glomerulus deformation} / 2 \] (2)

2.6. Statistical analysis

Values are reported as means ± standard deviations (SD), as indicated. The correlation between the ultrafine particle exposures and the mice kidney damages was evaluated by regression models using the Microsoft Excel 2016 software [21]. For all analyses, R² > 0.80 was considered statistically significant [9].

3. Results

3.1. Particle concentrations

Fig. 3 shows the example graphs of the measured ultrafine particle concentrations for M2 (as the representative of the whole samples). Particle concentration measurements were performed three times for each sample (1st measurement-3rd measurement). The blue dots show the 1st measurement results. The red dots indicate the 3rd measurement. By using Eq. (1), we calculate the total concentration. All graphs have the same trend, where the higher dose contains a higher concentration of ultrafine particles. In order to get a better understanding, Table 1 presents the total concentrations of ultrafine particles from M1-M5 calculated for a single exposure. From this table, we found that different motor sample produced ultrafine particles with the different concentration. The oldest model emits the highest concentration. The highest exposure concentration of C5 is 4.00 × 10⁵ particles/cm³ emitted by the sample of M1. The lowest one is 3.32 × 10⁵ particles/cm³ obtained from the sample of M5. As expected before, it was estimated that the older model of the engine emitted the highest concentration. The highest value of C1 exposure is 2.52 × 10⁵ particles/cm³ obtained from M2. The lowest one is 1.63 × 10⁵ particles/cm³ obtained from M4.
3.2. Damage percentage calculation

Fig. 4 shows the digital images of mice’s kidney after exposure to the concentration of C1, C2, C3, C4, and C5. The normal tubular epithelial cell is marked by a blue arrow. The abnormal tubular epithelial cells are presented by yellow arrows. The capsular space of the kidney is indicated by a black arrow (Bowman space).

Table 2 shows the physical damage percentages of kidney cells related to the varied dose concentration for different motorcycle samples. The kidney cell physical damage shows the similar trend depending on the dose concentration exposed to the mice for all motor samples. Exposing with more dose concentration results increasing of cell deformation. For the sample M1, the physical damage is (14 ± 4) % for the exposure dose concentration of C1, (14 ± 2) % for the exposure dose concentration of C2, (15 ± 3) % for the exposure dose concentration of C3, (18 ± 4) % for the exposure dose concentration of C4, and (23 ± 6) % for the exposure dose concentration of C5. The similar trends are found for the motor sample M2, M3, M4, and M5. The ultrafine particle exposure from the motor sample M2 with the dose concentration of C1, C2, C3, C4, and C5 cause the kidney cell physical damage.

![Fig. 2. The normal tubules and glomerulus (scale bar: 20 μm).](image1)

![Fig. 3. The measured ultrafine particle concentrations of the sample M2 with the dose concentration of C1, C2, C3, C4, and C5.](image2)
damage of (20 ± 3) %, (23 ± 3) %, (24 ± 3) %, (25 ± 4) %, and (28 ± 4) %. The increasing of the kidney cell physical damage shown for the sample M3 is (13 ± 5) % for C1, (14 ± 6) % for C2, (19 ± 5) % for C3, (19 ± 3) % for C4 and (22 ± 6) % for C5. For the sample M4, the kidney cell physical damage is (21 ± 3) %, (22 ± 4) %, (23 ± 5) %, (24 ± 4) %, and (26 ± 3) % for exposures to the mice with the concentration of C1, C2, C3, C4, and C5. Those results are strengthened by the kidney cell physical damages for the sample M5: (18 ± 5) % for C1, (18 ± 5) % for C2, (19 ± 4) % for C3, (20 ± 5) % for C4, and (21 ± 5) % for C5.

### 3.3. Dose particle concentrations vs damage percentages

The correlation between the particle concentration and the physical damage percentages (deformation levels) of mice kidney cells is presented in Fig. 5. A high concentration of the ultrafine particles in the motorcycle exhaust emissions causes large deformation levels of the kidney cells. Each motorcycle sample has a specific characteristic of the correlation between the kidney cell deformation and the total concentrations of ultrafine particles.

The correlation between the dose particle concentrations and the damage percentages is approached with the 2nd order polynomial function. The results show that the function is $y = 0.014 x^2 - 1.442x + 51.775$ for the sample M1; $y = -0.004 x^2 - 0.235x + 22.403$ for the sample M2; $y = -0.006 x^2 + 0.877x - 11.772$ for the sample M3; $y = 0.003 x^2 -0.132x + 22.972$ for the sample M4; and $y = -0.002 x^2 + 0.288x + 10.203$ for the sample M5 with the $R^2$ values are 0.99; 0.85; 0.86; 1.00; and 0.94 respectively. The significant correlation between the dose concentration of ultrafine particles in the motorcycle exhaust emission exposures to the mice and the kidney damage is shown by the $R^2$ values. Regarding Wardoyo et al. (2017) [9] that the correlation between two parameters with the $R^2$ values more than 0.80 considers that they have a significant correlation.

### Table 1

| Exposure Concentration | Calculated Particle Concentrations (x10^5 particles/cm^3) |
|------------------------|----------------------------------------------------------|
|                        | M1 | M2 | M3 | M4 | M5 |
| C1                     | 2.33 ± 0.27 | 2.52 ± 0.15 | 2.02 ± 0.05 | 1.63 ± 0.05 | 1.79 ± 0.18 |
| C2                     | 3.05 ± 0.15 | 2.98 ± 0.12 | 2.08 ± 0.09 | 2.26 ± 0.25 | 1.86 ± 0.08 |
| C3                     | 3.30 ± 0.05 | 2.98 ± 0.28 | 2.40 ± 0.46 | 2.62 ± 0.21 | 2.21 ± 0.01 |
| C4                     | 3.58 ± 0.27 | 3.63 ± 0.05 | 3.45 ± 0.20 | 3.02 ± 0.44 | 2.70 ± 0.33 |
| C5                     | 4.00 ± 0.75 | 3.86 ± 0.09 | 3.84 ± 0.20 | 3.40 ± 0.52 | 3.32 ± 0.33 |

### Table 2

| Motorcycle Samples | Physical Damage Percentages (%) |
|--------------------|----------------------------------|
|                    | C1       | C2       | C3       | C4       | C5       |
| M1                 | 14 ± 4   | 14 ± 2   | 15 ± 3   | 18 ± 4   | 23 ± 6   |
| M2                 | 20 ± 3   | 23 ± 3   | 24 ± 3   | 25 ± 4   | 28 ± 4   |
| M3                 | 13 ± 5   | 14 ± 6   | 19 ± 5   | 19 ± 3   | 22 ± 6   |
| M4                 | 21 ± 3   | 22 ± 4   | 23 ± 5   | 24 ± 4   | 26 ± 3   |
| M5                 | 18 ± 5   | 18 ± 5   | 19 ± 4   | 20 ± 5   | 21 ± 5   |

### 4. Discussion

Based on the results, we obtained a significant positive association between the total concentrations of ultrafine particles in the motorcycle exhaust emissions and the physical damages in mice kidneys. Analysis of the possible mechanism involved was based on the study of oxidative stress. The filtering system resulted in ultrafine particles with the diameter of 0.02–0.10 μm. These ultrafine particles allow us to analyze the cell deformation effects related to the size distribution and the particle concentration. In the present study, the different injected times show the different particles concentrations as the dose variances. These different concentrations can affect the different deformation level of the mice kidneys for all the samples. These persistent disruptive effects by the ultrafine particles exposures may be associated with the accumulation of them in the deeper organs.

The inhaled ultrafine particles can deposit into the lung and transfer through the bloodstream. They have capabilities to coagulate, agglomerate, and deposit in a surface area of a lung [22,23]. They are able to penetrate to the lower parts of the lung due to their small size [24]. When they penetrate to the lower part of the lung, they can transport through the bloodstream that may affect vital organs, such as the kidney. However, the molecular mechanisms underlying ultrafine particles induced the disruption of kidneys are not well understood. As we know, kidney plays an important role in ultrafine particle excretion.

![Fig. 4](image-url). The mice kidney images for the control and those after smoke exposure with the concentration of C1, C2, C3, C4, and C5 (scale bar: 20 μm).
When ultrafine particles are deposited and accumulated in kidney, they may affect the damage [26]. According to the results, the cell deformation level increased significantly as the higher ultrafine particle concentration. The exposures to ultrafine particles with the diameter of 0.02–0.10 μm can be used as a predictive parameter of the toxicity, as shown in another study in a case of the impacts on different cells [9]. Previous studies investigating the impacts of nanoparticles on kidney confirm the results of us. The effect of silica and copper oxide nanoparticles on kidney cells was observed [26,27]. Another study of zinc oxide nanoparticles influenced to the cellular viability of the human embryonic kidney cells [25]. The injection of lead selenide nanoparticles (nano PbSe) in rats showed a pathological effect in the kidney [28].

Fig. 5. The correlation between ultrafine particle concentrations and physical damage percentages (deformation level).

Regarding the effects of ultrafine particles in the kidneys, when ultrafine particles are inhaled by mice, they may cause oxidative stress in the kidney cells as biomarkers [29]. The toxicity of ultrafine particles was related to the interactions of these particles and biological system and cellular environment [26]. This toxic interaction may associate with the production of ROS (Reactive Oxygen Species). The production of ROS in kidney cells indeed was related to the exposure of ultrafine particles [27]. Several studies support that exposure of high-dose ultrafine particles indeed leads to human and animals organs damage [8,30]. Moreover, the imbalance between the production and the clearance rates of ROS in organ system leads to oxidative stress, heme degradation, and cardiac tissue injuries [31,32]. It has been reported that oxidative stress and ROS in cells are associated with the particle size, where the number of ROS in cells increased as the particle decreased [33]. As the impact of those ultrafine particles exposure, several immune parameters might be altered [34]. The disruption of immune parameters can be indicated by the changes of the neutrophils, eosinophils, and basophils that show a systematic inflammation in the kidney [35]. In this section, ROS, as the key of free radical in organs, can be linked to the systemic inflammation and diseases [36]. When the amount of ROS level is more than the anti-oxidant, there will be free radicals that get along with kidney tubules damages and cells viabilities [37]. As the impact, any systemic inflammation and oxidative stress can influence the initiation of kidney injuries [38], as well as in tubular and glomerular dysfunction due to oxidative stress [39].

Most studies on associations between high-dose exposure to particulate matters and mice were conducted using ambient particulate matter concentrations measured at a stationary monitoring state [11,40,41]. However, in this state the exposures are unvaried. In facts, there are so many kinds of ultrafine particles sources especially motorcycles. However, they can also generate secondary particles in certain particle distribution called ultrafine particles [42] in the different composition and concentrations [43]. Motorcycle exhaust gases also
contain hazardous particulate matters consisted of different chemical compounds [44]. According to literature, motorcycle exhaust particles can content of polycyclic aromatic hydrocarbons and volatile organic compounds [5,45]. The existences of volatile organic compounds and polycyclic aromatic hydrocarbons in ultrafine particles may react with the components of cells, causes the death of cells [46], and induces the oxidative stress. Polycyclic aromatic hydrocarbons and volatile organic compounds indeed become hazardous chemicals and toxic to human health due to their carcinogenic and even mutagenic characteristics [47–49]. Moreover, a study about the carcinogenic human health risk assessment using carcinogenic toxic equivalents of four commonly consumed smoked fish species from markets in Southern Nigeria confirmed that a high concentration of polycyclic aromatic hydrocarbons, such as benzo(a)pyrene (above the guideline value of 0.05 mg/kg), had potential carcinogenic risk from consumption [50].

5. Conclusions

In summary, there is a significant correlation between the ultrafine particle concentrations in the motorcycle exhaust emissions and the mice kidney cell physical damage in terms of the cell deformation. The high concentration of ultrafine particles in the motorcycle exhaust emissions causes more cell deformation. The correlation is found in the 2nd order polynomial function, with the R² values are more than 0.80.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Ethics approval

Ethical Clearance No: 541-KEP-UB, signed by Prof. Drh. Auliian’im, DES.

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