Full Length Research Paper

Exposure to air freshner and its distresses on the antioxidant biomarkers of male Wistar rats

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Air fresheners are widely used as a means of eliminating bad odour, albeit, it has been reported to be associated with some health risks. This work investigated the effect of acute exposure to air freshener on the antioxidant biomarkers of Wistar rats. Twenty-four Wistar rats were used for this study. The rats were divided into four groups of six rats each, labeled group 1, group 2, group 3, and group 4. Group 1, served as control which was not exposed to air freshener. Groups 2, 3 and 4 were exposed in a tightly enclosed cage to air freshener at 6, 9 and 12 h daily, respectively. The rats were sacrificed and blood collected in a sample bottle for analysis of the following antioxidants biomarkers; reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT) after 14 and 28 days of exposure. The results showed that the blood MDA concentration increased significantly at p≤0.05 with increase in exposure time, a concurrent decrease in catalase and reduced glutathione concentration was observed significantly in the exposed groups when compared to the control. The decrease in SOD was concentration dependent, although not statistically significant, while an inconsistent decrease in the GPx and GST concentrations were observed in exposed groups when compared with the control group. These findings suggest that exposure to air freshener increased oxidative stress, thereby posing potential health hazards to the regular consumers. This study therefore suggests a reduction in exposure to air fresheners as its adverse health effect is proportional to the length of exposure.

Key words: Air freshener, antioxidant, air pollution, oxidative stress.

INTRODUCTION

Air pollution occurs when harmful or quantities of substances including gases, particles and biological molecules are introduced into the earth's atmosphere. It is the introduction of unpleasant substances into the atmosphere (USEPA, 2017). These unpleasant substances can be chemicals, smoke, dust or allergens, a product of the combustion of fossil fuels (gas, coal and exhaust of vehicles) which are pollutants and are
detrimental to health causing skin, lung and eye irritation in a short term and blood disorder in a long term exposure (Mackenzie, 2016). Air pollution is associated with increased cardiovascular and pulmonary morbidity and mortality. The mechanisms of air pollution-induced health effects involve oxidative stress and inflammation. Oxidative stress can trigger redox-sensitive pathways that lead to different biological processes such as inflammation and cell death.

Air fresheners are consumer products that emit fragrance to provide an aroma to a space, to mask odor, or both (Mohammed and Yakasai, 2017). They are used at homes, cars, offices, churches, etc., to mask odors and create pleasant indoor space. However, despite its freshness, it can emit and generate a range of potentially hazardous air pollutants that can impair air quality. Air freshener contains hazardous chemicals like benzene, formaldehyde, phthalates, toluene, xylene, isopropyl alcohol, limonene, etc., which are air pollutants and are detrimental to health (BEUC, 2005; Alexander, 2017). Kim et al. (2015) reported some of the basic components of air freshener with their various adverse effects, thus volatile organic carbon (VOC) affects respiratory tract irritation, asthma, vomiting, and headache; carbon monoxide (CO) causes respiratory disorder symptoms, central nervous effect, nausea, chest pain and sickness; nitrogen dioxide (NO2) is lethal at high levels, potential chronic effects at low level, chronic respiratory symptoms. Exposure to VOC and ozone causes asthma, sensory irritation, respiratory symptoms and cardiac symptoms. In the study carried out by Fadeyi et al. (2013), they reported that when components emitted from air freshener reacts with ozone from several devices, it produces secondary pollutants namely oxidative products, ultrafine particles, formaldehydes, and secondary organic aerosols which affect human health both directly and indirectly. Odinga and Odu (2018) in their study, reported that exposure to air fresheners altered the bleeding time and blood electrolyte profile of albino rats.

Oxidative stress is an upset due to an imbalance between free radicals and antioxidants in the body. Free radicals are basically nasty little chemicals that go around stealing electrons off other molecules in the body that causes damage in the body (Willis and Lewis, 2017). Antioxidants are compounds which dispose, scavenge and suppress the formation of reactive oxygen species (ROS) or oppose their actions (Ogbuewu et al., 2010). They act by donating an electron to free radical without making themselves unstable, prevent the oxidation of the cells of the body that may lead to diseases by getting itself oxidized in place of the cell (El-Aal, 2012). The protective mechanisms of antioxidants serve to scavenge the free radicals. Different antioxidants act at different levels. They may prevent the initiation of chain reactions by removing free radicals; scavenge free radicals generated in chain reactions, thereby interrupting the chain sequence, remove peroxides, thus preventing further generation of ROS.

This study evaluated the effect of air freshener exposure on the antioxidant biomarkers of male Wistar rats.

MATERIALS AND METHODS

Ethical considerations

The internationally accepted National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) were observed (NIH, 2002).

Experimental animals

Twenty four male Wistar albino rats were obtained from the University of Port Harcourt and were divided into four groups of six albino rats each. They were allowed to acclimatize with unrestricted access to feed and water ad libitum. Group 1A and Group1B = control or non-exposed; Group 2A and 2B = exposed for 6 h per day; Group 3A and 3B = exposed for 9 h per day; Group 4A and 4B = exposed for 12 h per day.

All the experimental animals were housed in iron cages with many holes where temperature was maintained at room temperature.

The commercial air freshener used was purchased in Port Harcourt, Rivers State, Nigeria. After 14 days of acclimatization of the rats, they were exposed to gel air freshener inside a tightly closed room according to the method described by Atere and Osadolor (2017).

The animals were exposed to 40 g gel air freshener for 6, 9 and 12 h daily for the period of 14 and 28 days, respectively. The weight of the rats in each of the groups was recorded and their behaviors observed weekly. The control animals were kept under identical conditions without exposure to the air freshener. After 14 days exposure to air freshener, the rats were weighed to determine their final weight, three animals from each group were sacrificed via cardiac puncture and blood samples were collected by the use of 5 ml syringe, and dispensed into sterile plain bottle. Same process was repeated after 28 days of exposure of the remaining 3 experimental rats in each group.

The blood samples were taken to the laboratory for assay of the antioxidant biomarkers: reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT).

Procedure for assay

Catalase (CAT)

Catalase was calorimetrically determined using a catalase assay kit containing a stopping solution. The stop solution caused the complete stop of catalase activity. Catalase content of the samples reacted firstly with hydrogen peroxide to produce water and oxygen and the unconverted hydrogen peroxide reacted with OxiRed probe solution to produce a product which was measured at 570 nm. 12 μL of 1 mM fresh hydrogen peroxide solution was added to 40 μL serum samples or high control serum samples (HC sample), stopping solution was firstly added to HC serum samples, the
catalase activity was assayed. All samples were incubated at 25°C for 30 min, then 10 μL stop solution was added into sample vials. HC and standard samples already contained the stop solution. OxiRed probe solution was added to the samples and incubated at 25°C for 10 min. The absorbance at 570 nm was measured. A standard curve was prepared by adding 10 μL stop solution to 0, 2, 4, 6, 8 and 10 μL 1 mM hydrogen peroxide solution (Aebi, 1984).

**Malondialdehyde (MDA)**

MDA level in serum was assessed by the new colorimetric method of Satoh (1978). After the reaction of thiobarbituric acid with malondialdehyde, the reaction product was extracted in butanol and was measured.

**Superoxide dismutase (SOD)**

SOD levels in plasma were estimated by the method of Marklund and Marklund (1972), modified by Nandi and Chatterjee (1988). The ability of superoxide dismutase to inhibit the auto oxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for the SOD enzyme.

**Reduced glutathione (GSH)**

GSH levels in blood were assessed using 5,5-dithiobis-2-dinitrobenzoic acid (DTNB) by the method of Beutler et al. (1963).

**Glutathione peroxidase (GPx)**

GPx activity in blood was estimated by the method of Paglia and Valentine (1967) using H₂O₂ as a substrate.

**RESULTS**

The results obtained as shown in Tables 1 and 2 revealed that exposure to air freshener altered the antioxidant biomarkers adversely after 14 and 28 days of exposure, respectively.

**DISCUSSION**

The results obtained from this study revealed that experimental rats exposed to air freshener after 14 days as shown in Table 1 had an increase in the blood MDA concentration. MDAs are produced as a result of lipid peroxidation which is highly reactive and display marked biological effects, which depending on their concentration causes selective alterations in cell signaling, protein and DNA damage and cytotoxicity (Kota et al., 2013). A concurrent significant decrease (P≤0.05) in reduced glutathione level was also observed. The catalase level was seen to decrease with increase in exposure time, however, the decrease was not statistically significant.

Glutathione and catalase are the antioxidant defense system against oxidative stress, thus a decrease in their concentrations suggests an increase in oxidative stress of the experimental rats on exposure to air freshener (Halliwell, 1992). A concentration dependent decrease in SOD was also observed. SOD acts to help break down potentially harmful oxygen molecules in the cell which could lead to damage of the tissues (Carillon et al., 2014).

A decrease in the glutathione peroxidase level after 14 days exposure was seen to decrease for groups 2 and 3. However, an increase was observed in group 4. GPx catalyzes the reduction of hydrogen peroxide to water and oxygen as well as catalyzing the reduction of peroxide radicals to alcohols and oxygen, thus removing low levels of hydrogen peroxide that might damage the cell. Anderson and Anderson (1997) reported that mice sometimes develop a breathing pattern such that they inhaled a lesser amount of air fresheners at higher concentrations. This may explain the lack of dose dependency observed in this study. However, further studies are necessary to verify this assertion.

GST protects cellular macromolecules from attack by reactive electrophiles (Townsend and Tew, 2003). No statistical significant difference in the blood GST was observed, although there was an increasing variation compared to the control (Group 1).

After 28 days of exposure as shown in Table 2, same trend for 14 days exposure was observed. However, there was decreasing significant difference in reduced glutathione and malondialdehyde in group 3 exposed for 9 h and group 4 exposed for 12 h compared to the control (Group 1), while there was inconsistent decreasing

### Table 1. Antioxidant levels of experimental rats after 14 days of exposure to air freshener.

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH</th>
<th>GPx</th>
<th>GST</th>
<th>SOD</th>
<th>MDA</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>2.36±0.31</td>
<td>0.10±0.02</td>
<td>0.22±0.02</td>
<td>0.64±0.28</td>
<td>0.48±0.16</td>
<td>7.45±1.25</td>
</tr>
<tr>
<td>2A</td>
<td>2.30±0.51</td>
<td>0.07±0.01</td>
<td>0.36±0.02</td>
<td>0.50±0.28</td>
<td>0.57±0.23</td>
<td>7.06±2.12</td>
</tr>
<tr>
<td>3A</td>
<td>1.46±0.33</td>
<td>0.07±0.02</td>
<td>0.26±0.00</td>
<td>0.28±0.06</td>
<td>0.83±0.07</td>
<td>6.86±0.81</td>
</tr>
<tr>
<td>4A</td>
<td>1.08±0.51</td>
<td>0.10±0.02</td>
<td>0.27±0.03</td>
<td>0.32±0.05</td>
<td>0.70±0.05</td>
<td>5.44±0.39</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. Values with different superscripts show significant difference at the 0.05 level.
variation in glutathione peroxidase and catalase and increasing variation in glutathione-s-transferase compared to the control.

The decrease in blood antioxidant concentration following exposure to air freshener can be attributed to the volatile organic compounds (BEUC, 2005) leading to impairment in the free radical/antioxidant equilibrium which provokes a situation of oxidative stress and generally results from hyper production of reactive oxygen species (Yang et al., 2007). This alteration in the antioxidant defense makes the body system prone to diseases.

The results of this study are unswerving with previous reports which show that the components of air freshener decreased the antioxidant status of cellular constituents (Wang et al., 2013; Li et al., 2012).

**Conclusion**

Air freshener emits sweet fragrance, that has been welcomed all over the world to mask odours in homes, cars, offices, etc., regardless of the health risk associated to the unhealthy chemical components being absorbed or inhaled. This research reveals the alteration in the antioxidant defense due to air freshener inhalation.

**RECOMMENDATIONS**

The following recommendations can be made from this research.

1. Do not depend on air freshener to help eliminate odours, cleaning the environment by removing all sources of unpleasant odours is encouraged.
2. Use ventilation instead of air fresheners.
3. The use of plant with natural fragrance should be encouraged as an alternative to air freshener.
4. Further studies should be carried out on chronic exposure to adequate assess the health risk of air fresheners.
5. The Government Agency should ensure that all manufacturers of air freshener use less toxic chemicals and within permissible ranges to reduce health risk.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


**Table 2. Antioxidant levels of experimental rats after 28 days of exposure to air freshener.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH</td>
<td>GPX</td>
</tr>
<tr>
<td>1B</td>
<td>2.407±0.03</td>
<td>0.079±0.03</td>
</tr>
<tr>
<td>2B</td>
<td>2.367±0.16</td>
<td>0.059±0.01</td>
</tr>
<tr>
<td>3B</td>
<td>1.736±0.42</td>
<td>0.077±0.01</td>
</tr>
<tr>
<td>4B</td>
<td>1.350±0.20</td>
<td>0.068±0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. Values with different superscripts show significant difference at the 0.05 level.


