Nephro and Hepatotoxic Effect of Air-Freshener in Wistar Rats

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ABSTRACT

Aim: The present study was designed to examine the effect of inhaling ‘sunlight’ air-freshener on renal and hepatic indices of Wistar rats.

Methodology: Thirty Wistar rats were divided into three groups of ten each and kept in different rooms. Rats in group 1 were not exposed to any substance; those in groups 2 and 3 were exposed to sunlight air freshener for 8 hours daily for 28 days by inhalation. After 28 days of exposure, animals in group 3 were allowed to recover for 14 days. Throughout the experiment, all animals were fed ad libitum with standard feed and drinking water. At the end of the experiment, rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected via cardiac puncture. Hepatic and renal indices were determined using standard methods.

Results: Air-freshener significantly (p<0.05) increased hepatic and renal indices investigated when compared to those in animals in the control group. The effect on hepatic indices was reversed when...
animals were allowed to recover for 7 days after 28 days exposure. However, the effect on renal indices was sustained after 7 days recovery period.

**Conclusion:** The results of this study suggest that frequent exposure to air-fresheners poses a dangerous risk to the health of the liver and kidney. This is due to the presence of toxic chemicals such as the volatile organic compounds, which are toxic even at very low concentration, thus proper awareness should be conducted to educate consumers on the risk associated with the frequent use of this product.

**Keywords:** Air-freshener; hepatotoxicity; nephrotoxicity; pollutant.

### 1. INTRODUCTION

Air-fresheners are consumer products that emit fragrance to introduce an aroma into the air, to mask an odour, or both [1]. They have become a staple in many Nigerian homes and offices, marketed with the promise of creating a clean, healthy, and pleasurably aroma that eliminate or mask bad odours in the space. Nonetheless, consumers are unaware that the release of this fragrance can emit and generate a wide range of potentially hazardous chemicals that can pollute the air and pose a threat to human health. Air fresheners contain many chemicals that are not revealed on the product label as manufacturers are not required to disclose all ingredients [2]. These chemicals could be allergens, irritants, or even toxic [3]. Air-fresheners come in diverse forms, including incense, sprays, gels, electric diffusers, scented candles, and aerosol liquid wick [4].

Past studies have determined air fresheners to be sources of various volatile organic compounds (VOCs) found in indoor environments [5-7]. The major chemical constituents found in air fresheners include volatile organic compounds (VOCs), such as benzene, toluene, ethylene and limonene [8], which are known to add to environmental pollution [1]. Numerous studies have shown that VOCs cause a wide range of adverse effects such as headache, breathing difficulties, asthma attack, mucosal symptoms, drowsiness, dizziness, tremor and coma [1,4]. Sunlight air freshener is the most common air freshener popularly used in Nigeria [9]. Recently, Airaodion et al. [9] reported that air-freshener induced oxidative stress and adversely affects immunity. It has also been reported that air fresheners had a negative impact on male fertility [10]. The present study was designed to examine the effect of inhaling ‘sunlight’ air freshener on renal and hepatic indices of Wistar rats.

### 2. MATERIALS AND METHODS

#### 2.1 Collection of Air Freshener

Sunlight air-freshener was purchased in a super market at Douglas area of Owerri, Imo State, Nigeria and was kept at room temperature before and during the experiment.

![Fig. 1. Sunlight air freshener [9]](image)

#### 2.2 Experimental Design and Animal Treatment

Thirty Wistar rats weighing between 160 and 190 g were used for this study. They were acclimatized for seven (7) days to laboratory conditions before the commencement of the experiment. During this period, they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institute of Health. After the 7 days acclimatization period, the animals were weighed and recorded and were divided into three groups of ten each and kept in different rooms. Rats in
group 1 were not exposed to any substance, those in groups 2 and 3 were exposed to sunlight air freshener by inhalation for 8 hours daily for 28 days following the method of [11]. After the 28 days of exposure, animals in group 3 were allowed to recover for 14 days. Throughout the experiment, all animals were fed ad libitum with standard feed and drinking water. At the end of the experiment, rats were weighed and recorded. They were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected via cardiac puncture.

2.3 Determination of Hepatic Indices
Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were determined using Randox commercial Enzyme kits according to the method of Reitman and Frankel [12]. Alkaline Phosphatase (ALP) activity was determined by Phenolphthalein Monophosphate method described by Babson et al. [13].

2.4 Determination of Renal Indices
Creatinine concentration was determined using Jaffe reaction described by Toora and Rejagopal [14]. Urea concentration was determined using a Randox Commercial Kit based on the methods of Fesus et al. [15].

2.5 Statistical Analysis
Data were subjected to analysis using Analysis of Variance (ANOVA) with the aid of Graph Pad Prism. Data from each parameter was expressed as mean ± standard error of the mean (SEM). Data were considered to be significantly different at 95% confidence level (P < 0.05).

3. RESULTS
The results of the effect of exposure of sunlight air freshener on aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine and urea are presented in Figs. 2-6 respectively.

![Fig. 2. Effect of air-freshener on the activity of aspartate aminotransferase (AST) in animals after 28 days of exposure](image-url)
Fig. 3. Effect of air-freshener on the activity of alanine aminotransferase (ALT) in animals after 28 days of exposure

Fig. 4. Effect of air-freshener on the activity of alkaline phosphatase (ALP) in animals after 28 days of exposure
4. DISCUSSION

Every organ can elicit a specific pattern of enzyme release, which remains unelucidated. Specifically, above-normal plasma enzyme activities are considered as diagnostic features for several diseases [16]. Liver tissue damage due to toxic compounds is associated with its...
detoxification function. Air-freshener contains a variety of volatile organic compounds (VOCs) such as formaldehyde with the potential to damage the liver [17].

The air-freshener used in this study was observed to significantly increase the activity of AST when compared with those in the control group at p<0.05 (Fig. 2). This increase might be an indication of hepatotoxicity of air-freshener as AST is a biomarker enzyme found to be abundant in cases where the liver might be damage [18,19]. However, the activity of AST in group 3 was slightly decreased when compared to those in group 2. This decrease is attributed to the fact that these group had been allowed to recover for 7 after 28 days of exposure. This suggests that recovery can significantly reduce the effects of toxicity if the hepatic tissues affected are given time to heal.

Similarly, the activity of ALT was significantly (p<0.05) increased in animals exposed to the air-freshener when compared with those in control animals. ALT levels of animals allowed to recover were reduced when compared with those not allowed to recover (Fig. 3). The distribution and relative tissue concentration of ALT is similar but importantly different. The highest activity is found in the liver, followed by kidney, myocardium, skeletal muscle, pancreas, spleen, lung, and erythrocyte [20]. ALT activity is found in the cytosol, unlike AST which is both cytosolic (20% of total activity) and mitochondrial (80% of total activity) [18,21]. The release of mitochondrial enzymes from the liver is considered to provide strong evidence for hepatic necrosis [22]. AST is also found in other organs such as the heart and skeletal muscle, while ALT has low concentrations in the skeletal muscle and kidney, and is chiefly produced in the hepatocytes [21,23]. Release of liver mitochondrial enzymes is considered as a strong evidence for hepatic necrosis, which is associated with increased production of Reactive Oxygen Species (ROS), often leading to greater hepatic lipid peroxidation [24]. Air-freshener has been reported to induce oxidative stress by increasing the production of ROS [9]. Thus, its hepatotoxic effect observed in this study might be due to its ability to induce oxidative stress. This is in agreement with the findings of Tang et al. [25], who reported that liver damage could be due to increased production of ROS and lipid peroxidation resulting from formaldehyde exposure. It is also consistent with the findings of Airadion et al. [26] who reported the nephro- and hepato-toxicity of common household insecticides used in Nigeria. Air-freshener had no significant effect on the activity of ALT on animals allowed to recover for 7 days after 28 days of exposure. This might be suggestive that the toxic effect of air-freshener on the activity of ALT is reversible.

ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissues. It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile canaliculi, located in the plasma membrane [26,27]. The significant (p<0.05) increase observed in the activity of ALP in animals exposed to air fresheners when compared with those in control animals could be attributed to the formaldehyde component in it. The result of this study is consistent with the findings of Beall and Ulsamer [28], who reported that formaldehyde exposure is associated with hepatotoxicity. Although the hepatic changes are generally not extensive and can be reversible following acute exposure. Repeated exposures cause more serious progressive damage. This finding is progressive and consistent with other findings [29-32] who respectively reported a significant increase of liver enzymes, ALT and AST in similar studies of animals exposed to air fresheners. Since ALP hydrolyses phosphate monoesters, its significant increase in animals exposed to air-freshener could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver [33]. Consequently, this may adversely affect the facilitation of the transfer of metabolites across the cell membrane of animals exposed to air-freshener.

Air-freshener used in this study significantly (p<0.05) increased the concentrations of creatinine and urea when compared with those of animals in the control group (Figs. 5 and 6 respectively). Urea is a major nitrogenous end product of protein and amino acid catabolism and creatinine is a breakdown product of creatinine phosphate in the muscle [34]. They are excreted by the kidney. The significant rise in serum creatinine observed in this study most probably represents the increased production of creatinine to meet the energy demand following severe oxidative stress caused by the chemical components of the air-freshener.
Urea and creatinine are good indicators of a normal functioning kidney and an increase in the serum are indications of kidney dysfunction as they are widely accepted and commonest parameters to assess renal functions [35]. This result is consistent with the findings of Ajayi et al. [35] who reported that serum urea and creatinine significantly increased in animals exposed to air fresheners. These results suggest that kidney functions were seriously impaired by the components of air-fresheners. This may increase the incidence of chronic kidney disease arising from nephrotoxicity of air fresheners and its ability to damage the kidney. It is worrisome to note that renal toxicity induced by air-freshener exposure in this study was sustained even after 7 days recovery period.

5. CONCLUSION

The results of this study suggest that frequent exposure to air-fresheners poses a dangerous risk to the health of the liver and kidney. This is due to the presence of toxic chemicals such as the volatile organic compounds, which are toxic even at very low concentration, thus proper awareness should be conducted to educate consumers on the risk associated with the frequent use of this product.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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