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Biochemical Assessment of Oxidative Stress Markers in Rats Following Exposure to Spray and Scented Candle Air Fresheners

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ABSTRACT

Air freshener and scented candles are widely used to improve indoor air quality or eliminate unpleasant odors, but these consumer products release volatile organic compound (VOCs), particulate matter, and other chemicals that can adversely affect public health. This study evaluated the effects of air freshener spray and scented candle using a locally manufactured inhalation exposure chamber in Baghdad. Effects in rats were assessed with particular focus on the oxidative stress. A total of (40) healthy Wistar male rats, weighing between (180-200) gm, were randomly divided into 4 groups, each group containing 10 rats. The first group was exposed to air freshener spray, the second group exposed to scented candles, the third group exposed to mixture of air freshener spray and scented candle and the fourth group exposed to fresh air only. The inhalation exposure periods were (10, 20, 30) days for each group. The total exposure time was one hour daily, while the exposure time to the substance itself was estimated to be 15 minutes. Blood samples were collected from each rat, and serum was separated for biochemical analysis. Oxidative stress biomarkers (SOD, GPX, and LPO) were measured using ELISA kits, while GSH was determined manually using the Ellman method. The results demonstrated that exposure to the air freshener led to significant increase in GPX levels, while GSH concentrations were notably reduced. Additionally, the activities of antioxidant enzymes SOD and LPO were elevated compared to the control group. These findings indicate that such emissions can disrupt redox balance and may have toxic effects.

HIGHLIGHTS

A local inhalation chamber was designed to simulate real indoor exposure. VOC emitting sprays and candles altered oxidative stress biomarkers in rats. GPX and LPO levels increased, while GSH significantly decreased after exposure. Air fresheners disrupted redox balance, indicating potential toxic effects.

1. INTRODUCTION

People are generally aware that polluted outdoor air can harm human health, but many fail to overlook the fact that indoor air pollution can be equally, if not more, detrimental. The Environmental Protection Agency (EPA) monitors and regulates air quality both outdoors and indoors. According to the EPA, the concentration of indoor air pollutants can be up to 100 times higher than that outdoor air pollutants. This makes indoor air quality a critical concern for public health, especially considering that individuals spend nearly 90% of their time within enclosed environments (Seguel et al., 2017). Common indoor pollutants, such as carbon monoxide (CO), ozone (O_3), particulate matter (PM), and volatile organic compounds (VOCs) are often found at concentrations significantly higher than those measured outdoors, posing substantial risks to human health (Cheek et al., 2021). Numerous studies have highlighted the hazardous nature of VOCs emphasizing their capacity to induce adverse health effects and contribute to indoor air pollution (Hammod et al., 2020). Indoor exposure to VOCs has been associated with non-carcinogenic and carcinogenic health risks. Recent investigations in small scale workplaces have shown significant occupational exposure to compounds such as benzene, ethylbenzene and other compounds exhibiting potential carcinogenic effects. Moreover,

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long term occupational exposure to VOCs particularly formaldehyde and acetaldehyde has been reported to exceed acceptable hazard thresholds posing both non carcinogenic and carcinogenic risks (Seo et al., 2025; Choi et al., 2023). Indoor environments contain a complex mixture of pollutants originating from both outdoor and indoor sources. Outdoor contaminants primarily derive from vehicular traffic and industrial emissions that can infiltrate through natural or mechanical ventilation systems. In addition, numerous indoor sources contribute to pollutant levels, including the combustion of fuels, coal, tobacco products, and candles, as well as emissions from building materials, and household cleaning products, and occupant behaviors such as smoking (Cincinelli and Martellini, 2017).

Air fresheners are extensively used across various indoor settings within modern society. All forms of air fresheners (such as gels, sprays, oils, solids, and diffusers), including those marketed as "green" or "organic" products, have been found to release VOCs that may pose health risks. Among these, one major compound, d-limonene has been reported at an average concentration of 6.78 µg/m^3 in area using air fresheners compared to 0.84 µg/m^3 in non-using areas with reduction up to 96% after discontinuation (Goodman et al., 2020). The increasing popularity of scented candles has prompted scientific interest due to concerns over their contribution to indoor air pollution resulting emissions. These products emit a complex mixture of aromatic and non-aromatic substances, including volatile organic compounds (VOCs and SVOCs), as well as particulate matter. For example, Yun et al. (2025) reported that PM levels increased by 1.52 times during candle burning (Yun et al., 2025; Adamowicz et al., 2019). Prolonged or repeated inhalation of these substances, especially volatile organic compounds (VOCs), has been associated with irritation of the eyes, nose, and throat, as well as symptoms such as nausea, difficulty in breathing, and potential damage to the central nervous system and other organs (Singh et al., 2023).

Oxygen plays a crucial role in aerobic metabolism due to its high reactivity; however, this same property also contributes to the generation of free radicals that oxidize biological molecules. These reactive species can also serve as part of the body's defense mechanisms against invading pathogens. To limit potential cellular damage, organisms maintain tight regulation over the production of reactive oxygen species (ROS). Antioxidants can act as key molecular defense systems, and variations in their levels are often used as biomarkers to assess of environmental stress (Hellou et al., 2012; Alfahdawi et al., 2023). In fact, pollutants such as particulate matter, ozone, nitrogen oxides, and transition metals are either strong oxidants themselves or capable of generating reactive oxygen species. The resulting oxidative stress can activate redox-sensitive signaling pathways, which, in turn initiate biological processes including inflammation and programmed cell death (Lodovici and Bigagli, 2011).

Endogenous antioxidant enzymes, such as glutathione and superoxide dismutase. Play essential roles in protecting humans from oxidative damage; however, variations in antioxidant defense systems exist across species. Oxidative stress arises from an imbalance between the production of ROS, metal ion homeostasis, and the body's antioxidant defenses systems (Banks and Rhea, 2021). Recent studies have shown that the activities of key antioxidant enzymes—glutathione peroxidase, superoxide dismutase, and catalase—in the blood are strongly but inversely associated with an increased risk of developing coronary artery disease. Moreover, elevated levels of oxidized DNA in blood cells have been linked to pollution exposure in several cross-sectional studies (Khalid and Rabee, 2025).

Superoxide dismutase is a vital antioxidant enzyme that helps protect the body against the harmful effects of oxidative stress (Fakhri and Al-Ani, 2025). Glutathione peroxidase is also commonly measured in epidemiological studies to assess oxidative stress levels. Although numerous studies have attempted to determine the link between exposure to ambient particulate air pollution respiratory diseases and other health outcomes, the relationship between ambient particulate matter and major oxidative stress biomarkers remains inconclusive (Li et al., 2020).

This study was conducted to assess the effects of indoor air pollution associated with spray air fresheners and scented candles on some biological parameters in rats. To highlight the possible health hazards linked to such exposure within indoor settings.

2. METHODOLOGY

2.1 Collection of air freshener and scented candle samples

For this study, two (2) samples, non-combustible and combustible samples were conveniently selected at random from the air freshener sections of various stores representing different

manufacturers and sellers in Baghdad City. Both products are commercially available and can be used by consumers. However, the same brands and product types are widely distributed throughout Iraq and in internationally markets. The types of air fresheners sampled were one spray air freshener and one scented candle. All air fresheners were kept in their original containers and maintained at ambient temperature until analysis. The key features of the investigated sample are summarized in Table 1. Sample were collected from October 2023 to December 2023.

Table 1. Principal features of the investigated air freshener

Samples	Container	Status fragrance	Origin	Weight
Air freshener spray	Plastic spray can	Clean	Turkiye	500 mL
Scented candle	Glass beaker	Twisted Peppermint (Cool Peppermint, Sugared Snow, Vanilla Butter cream)	USA (including US and non-US parts)	411 g

The study was conducted with the assistance of the animal house unit at the Biotechnology Research Center, University of AL-Nahrain, from January 2024 to June 2024.

2.2 The inhalation exposure unit

The inhalation exposure unit is dynamic system, whole-body chamber commonly used in studies involving prolonged exposure and large numbers of laboratory animals. Designed according to the WHO specifications as described by (Al-Easawi, 2015; WHO, 1978).

2.2.1 The inhalation unit manufacturing

The laboratory unit used in this study consists of the following components as shown in Figure 1 below:

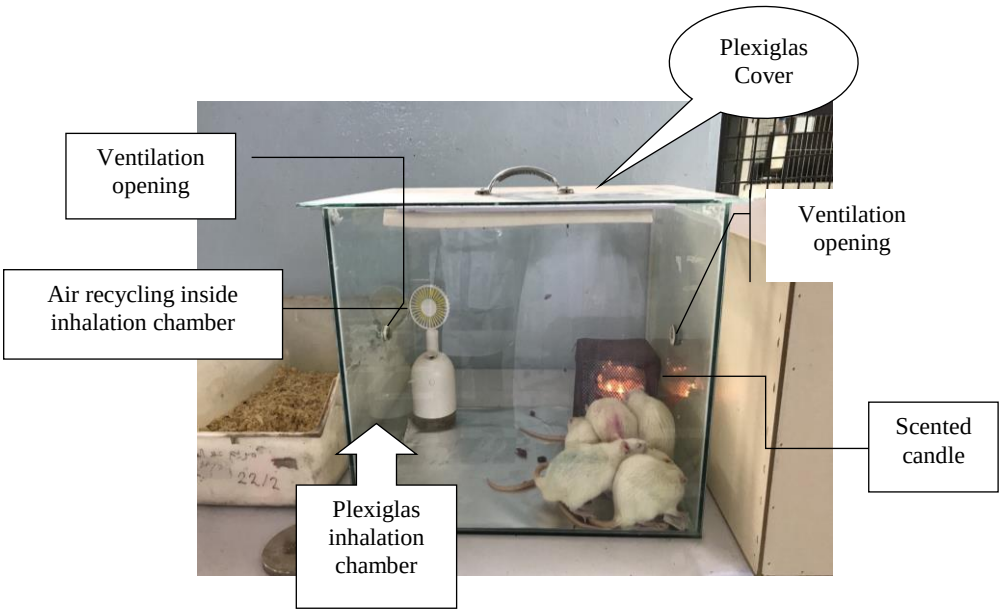

Figure 1. The locally manufactured inhalation exposure unit and its components

The features and design of the inhalation exposure chamber followed the description by Al-Easawi (2015): a 5 mm transparent Plexiglas chamber, manufactured locally with the following dimensions (50 cm length × 40 cm width × 40 cm height) of 80 Lt. Capacity (1) The chamber contained three openings: input, output and ventilation. The input and output openings were located on both sides of the chamber, while ventilation opening is a narrow strip along the top cover of the chamber. To ensure homogeneous distribution of the emitted vapors inside the exposure chamber, a small internal fan was installed to circulate the air during exposure sessions, promoting even mixing of emissions from both the air freshener and scented candle (Figure 1). To minimize carryover effects between different exposure types (spray, candle, and mixed), the chamber was thoroughly cleaned and ventilated with fresh air after each exposure session, and the next exposure was initiated only after confirming that baseline air conditions were restored. The chamber was equipped with a thermometer to monitor temperature during the

inhalation exposure experiments. Animal wastes fell onto removable aluminum foil covering the chamber floor, facilitating cleaning after each daily exposure.

2.3 Experimental animal groups

Forty (40) male Wistar rats, weighing between 180-200 g, were used in this study. They were acclimated to laboratory conditions for seven days before the experiment. They were purchased from the National Center for Drug Control and Research. After acclimatization period, the rats were weighed and the data recorded then divided into four groups; each ten rats in each group, including exposed and control groups. The rats were kept in a polypropylene cages (30 cm × 15 cm × 15 cm diameter) covered with wire grid lids and maintained at a temperature range of 27-29°C. Cages were kept under standard animal house conditions. Cages were kept under standard animal house conditions where the humidity ranged between 56-58% and 12±2 h (light/day). Rodent diet and drinking water were available *ad libitum*, except during inhalation exposure.

All experimental procedures were carried out in compliance with ethical standards for animal research. Approval from the ethics committee was obtained prior to conducting the study (Baghdad University/College of Science/Department of Biology/Ref.: CSEC/1123/0123 on November 26, 2023).

2.4 Experiment design

Forty (40) rats were randomly divided into four groups (30 exposed and 10 controls) according to LT50, with each group containing ten animals as follows:

Group (A): 10 rats were exposed to inhaled air freshener spray (2.5 mL). Exposure lasted 60 minutes daily, beginning 15 minutes after opening the freshener.

Group (B): 10 rats were exposed to inhaled scented candle. Exposure lasted 60 minutes daily, beginning 15 minutes after lighting the candle.

Group (C): 10 rats were exposed to fresh air only (control group).

Group (D): 10 rats were exposed to a mixture of air freshener spray (2.5 mL) and scented candle at the same time. Exposure lasted 60 minutes daily, beginning 15 minutes after opening the air freshener and lighting the candle.

For each exposure session 2.5 mL of the air freshener product was placed in an electric diffuser positioned inside the inhalation chamber. After introducing the rats the diffuser was turned on to actively emit the product and the chamber was sealed for 15 minutes to maintain a stable exposure atmosphere. The chamber was then kept closed for the remainder of the 60 minutes exposure period to ensure consistent inhalation conditions.

Each group was further divided into three subgroups based on exposure duration: three rats for 10 days, three rats for 20 days, and four rats for 30 days.

2.5 Animal's blood test

The rats in their various groups were exposed for 10, 20, and 30 days, and three rats from each group were sacrificed at the end of their respective exposure periods. Blood samples were collected for biochemical analysis of oxidative stress markers.

Blood samples were obtained via cardiac puncture using 1 mL Syringes and placed into Gel tubes, to determine the following: glutathione peroxidase (GPX), lipid peroxidase (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH). The blood samples were placed in gel tubes and left to coagulate at room temperature for 10-20 minutes. Serum was then separated by centrifugation for 20 minutes at 2,000-3,000 rounds per minute (rpm). The supernatant without sediment was collected in the eppendorf tube, and stored at -20°C until assayed (Abu Ghazal et al., 2023).

2.6 Oxidative stress parameters

2.6.1 Determination of Serum GSH concentration

The serum concentration of GSH was assessed using the Ellman method (Ellman, 1959; Al-Badry et al., 2023).

To prepare the reagents required for GSH estimation, phosphate buffer solutions were first prepared.

(A) Phosphate (H_2NaPO_4) was prepared at a concentration of 0.2 M by dissolving 0.2 g of the salt in 100 mL of distilled water.

(B) Disodium phosphate (HNa_2PO_4) was prepared at a concentration of 0.2 M by dissolving 0.2 g of the salt in 100 mL of distilled water.

Reagent A: (Phosphate buffer 0.2 M; pH=7) was prepared by mixing 41 mL of B with 9 mL of solution A and adjusting the volume to 100 mL using distilled water, and pH was corrected before and after dilution.

Reagent B: (Phosphate buffer 0.2 M; pH=8) was formed by combining 5 mL of solution A with 45 mL

of solution B, adjusted to 100 mL with distilled water, and pH was corrected before and after dilution.

Reagent C (DTNB reagent): was prepared by dissolving 39.6 mg of DTNB in 10 mL of reagent A, with a small amount of Na_2CO_3 added.

For the assay, 20 µL of serum was diluted with 1,000 µL of distilled water, followed by the addition of 1,000 µL of reagent B and mixed well. From this mixture, 1,500 µL was transferred into new tube and mixed with 200 µL of reagent C. The reaction mixture was incubated at 37°C for 60 minutes. A blank sample was prepared similarly, replacing serum with distilled water. Absorbance was read at λ=42 nm. The concentration of GSH was calculated according to the following formula Equation 1:

$$\text{GSH con. inserum}\ \frac{\mu mol}{L} = (T - B) \times \frac{df}{\varepsilon} \times 10 \qquad (1)$$

Where; T: test absorbance; ε: extinction coefficient=13,600 $M^{-1}.cm^{-1}$; B: blank absorbance =0.030 in this test; df: dilution factor=102.

2.6.2 Determination of serum SOD, LPO and GPX concentration

Serum levels of superoxide dismutase (SOD), lipid peroxidation (LPO), and glutathione peroxidase (GP_X) were measured using ELISA Kit: SOD: BT LAB, China, Catalog No. E0168Ra, LPO: BT LAB, China, Catalog No. E0285Ra, and GPX: BT LAB, China, Catalog No. E1242Ra. All procedures were performed according to the manufacturer's instructions (Ahmed and Yenzeel, 2017).

2.7 Statistical analysis

The statistical packages for the social sciences (SPSS) (2019) program was used to analyze the effect of different groups and factors on the study parameters. The least significant difference (LSD) test was used to compare means between groups, with significance considered at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Biomarkers of oxidative stress

3.1.1 Glutathione peroxidase (GPX)

The highest mean value (1745.48±53.99 pg/mL) was recorded in rats exposed to the spray air freshener after 30 days, while the minimum mean value (833.67±35.09 pg/mL) was observed in the control group after 10 days; (Table 2). Statistically, analysis showed no significant differences over time within each group, as GPX levels remained relatively stable from day 10 to day 30. However, significant differences were observed between the groups on each day, with the exposed groups showing much higher GPX levels than control. This is confirmed by the LSD values and ($p\leq0.05$).

Table 2. Mean value±SE of GPX levels pg/mL in experimental animals exposed to air freshener spray, scented candle, mixed, and control group after10, 20, and 30 days

Group	Mean±SE of GPX pg/mL			L.S.D.
	10 day	20 day	30 day	
Air fresheners (A)	1,717.91±253.73 A a	1,720.69±304.92 A a	1,745.48±53.99 A a	64.39 NS
Scented candle (B)	1,558.77±82.58 B a	1,611.55±203.31 AB a	1,671.04±94.75 A a	123.67 NS
Mix (D)	1,665.05±33.40 AB a	1,556.63±124.81 B a	1,656.85±94.82 A a	133.84 NS
Control (C)	833.67±35.09 C a	971.04±98.39 C a	955.64±106.36 B a	139.02 NS
L.S.D.	147.47 *	163.58 *	141.66 *	---

Different capital letters in column and small letters in row indicate significant differences * ($p\leq0.05$). NS: Non-significant.

The present study showed a significant increase in serum GPX levels in rats exposed to air fresheners and scented candles compared to the control group. The observed elevation in GPX levels may reflect a physiological adjustment by the antioxidant defense system in response to ongoing oxidative stress caused by VOCs exposure. These findings are consistent with the observation of Airaodion et al. (2020a), who reported a significant increase in GPX levels after 28 days of daily exposure to sunlight air freshener in rats, suggesting a compensatory antioxidant response to oxidative challenge. The similarity may be attributed to comparable exposure duration and inhalation route. However, the current study differs from that of

Gabriel-Brisibe et al. (2020), who reported a decrease in GPX levels after up to 28 days of exposure. Although the reduction was not statistically significant, it was interpreted as an early depletion of antioxidant capacity under acute oxidative stress conditions. This contrast may be explained by differences in the type of air freshener used and variations in ELISA kits. It is worth nothing that while some studies measured GPX enzymatic activity (U/mg protein), the present study assessed its concentration in pg/mL, which might also explain the observed discrepancy.

Afighor et al. (2019) demonstrated that VOCs present in air fresheners rapidly react with O_3 to generate more harmful oxidant molecules and related by-products. Moreover, oxidative stress has been linked to neuroinflammation, which is associated with the degeneration of neuronal pathways involved in learning and memory. In this context, the effects of long-term air freshener exposure have gained growing scientific attention. These changes are likely associated with the oxidative stress caused by VOCs exposure.

3.1.2 Lipid peroxidase (LPO)

Table 3 shows that LPO levels were highest in the mixed exposure group across all days, especially on day 10 (23.81±1.15 nmoL/mL). The air freshener and scented candle groups also showed elevated LPO levels, with slight variations over days. In contrast, the control group consistentently had the lowest LPO values throughout the experiment during 10, 20, and 30 days (10.4±2.33 nmoL/mL; 8.38±0.64 nmoL/mL; 11.17±1.28 nmoL/mL) respectively. Statistical rows analysis showed no significant differences in LPO levels over time within each group analysis between days 10, 20, and 30. This suggests that LPO levels remained stable within each group throughout the exposure period. In contrast, comparison between groups (columns) revealed significant differences ($p \leq 0.05$).

Table 3. Mean value±SE of LPO nmoL\mL in experimental animals exposed to air freshener spray, scented candle, mixed and control group after10, 20, and 30 days

Group	Mean±SE of LPO nmoL/mL			L.S.D.
	10 day	20 day	30 day	
Air fresheners (A)	18.83±0.63 B a	21.29±1.57 A a	22.60±0.59 A a	4.01 NS
Scented candle (B)	20.07±1.22 AB a	19.44±2.10 A a	19.67±2.43 A a	2.69 NS
Mix (D)	23.81±1.15 A a	21.92±1.12 A a	22.94±1.27 A a	2.94 NS
Control (C)	10.45±2.33 C a	8.38±0.64 B a	11.17±1.28 B a	3.02 NS
L.S.D.	4.51 *	5.06 *	4.72 *	---

Different capital letters in column and small letters in row indicate significant differences * ($p \leq 0.05$). NS: Non-significant.

In the present study, LPO levels, measured in nmoL/mL, showed a clear increasing trend in rats exposed to air freshener, scented candle and their combination, compared to the control. This suggests that exposure to airborne chemical compounds induces oxidative stress, as evidenced by elevated serum LPO levels. Although hepatic enzymes were not assessed in the present study, the observed elevation in serum LPO levels following air freshener exposure may serve as an early indicator of oxidative stress potentially affecting hepatic tissues. This aligns with Airaodion et al. (2020b), who reported that air fresheners induce oxidative stress through increasing reactive oxygen species and LPO contributing to hepatotoxicity, particularly in association with formaldehyde exposure.

According to Yakasai and Mohammed (2022), air fresheners are made from hydrocarbons, along with other ingredients. Inhaling these hydrocarbons has been found to be toxic to the liver, as their metabolism generates free radicals that react with macromolecules in the liver, ultimately causing lipid peroxidation. The formation of active metabolites, together with accompanying LPO, appears to be among the primary mechanisms by which air fresheners may damage liver cells. These finding are consistent with the current study, which reported a significant increase in serum LPO levels following exposure to both air freshener and scented candles. This similarity supports the

notion that the oxidative stress by volatile compounds emitted from these products may represent a common underlying mechanism.

Airaodion et al. (2020c) reported that exposure to air fresheners resulted in increased malondialdehyde levels in rats, accompanied by increased antioxidant enzyme activity compared to the control group. This increase may indicate potential disruption of the body's antioxidant defense mechanisms, which is consistent with the findings of the present study. In additional, widespread tissue damage caused by free radicals-driven LPO may compromise the integrity of cell membranes, ultimate leading to decreased membrane fluidity (Oyenihi et al., 2016). Previous studies have also shown that prolonged exposure to air fresheners can increase malondialdehyde levels while reducing endogenous antioxidant molecules in the brains of mice (Afighor et al., 2019).

3.1.3 Superoxide dismutase (SOD)

The highest serum SOD level was observed in the air freshener group (5.54±0.17 ng/mL), while the lowest value was recorded in the control group across 10, 20, and 30 days. Statistical analysis showed no significant differences over time within each group (row analysis). However, comparison between groups (columns analysis) revealed significant differences only on day 10 ($p \leq 0.05$) (Table 4).

Table 4. Mean value±SE of SOD levels ng/mL in experimental animals exposed to air freshener spray, scented candle, mixed exposure, and control group after 10, 20, and 30 days

Group	Mean±SE of SOD ng/mL			L.S.D.
	10 day	20 day	30 day	
Air fresheners (A)	5.53±0.54 A a	5.11±0.67 A a	5.54±0.17 A a	1.02 NS
Scented candle (B)	5.38±0.26 A a	5.18±0.03 A a	4.49±0.51 A a	1.37 NS
Mix (D)	5.35±0.50 A a	4.12±0.39 A a	5.24±0.29 A a	1.31 NS
Control (C)	3.16±0.47 B a	3.57±0.34 A a	3.76±0.23 A a	1.27 NS
L.S.D.	1.42*	2.031 NS	2.178 NS	---

Different capital letters in column and small letters in row indicate significant differences * ($p \leq 0.05$). NS: Non-significant.

Supporting our results, Kim et al. (2021) found that air freshener exposure increased the expression of oxidative stress- related proteins, such as SOD, in mice with NAFLD. This suggests that air freshener can trigger an antioxidant response under oxidative stress conditions. While their study measured SOD in liver tissue of diseased mice, and ours assessed serum levels in healthy rats. Despite these differences, both observed elevated SOD after exposure. The difference in sample type and health status may explain why the increase in our study was not statistically significant.

Air freshener exposure led to a marked increase in antioxidant enzyme activity compared to the control group. SOD plays a role in mitigating the harmful effects of free radical attacks, as it is the only enzymatic system capable of converting (O_2^-) into oxygen and hydrogen peroxide (H_2O_2). This function is essential for protecting cells against oxidative stress. These radicals are known to damage polyunsaturated fatty acids and proteins (Airaodion et al., 2020a).

3.1.4 Reduced glutathione (GSH)

The control group consistently showed the highest mean of GSH levels (191.00±11.00 µmol/L; 183.00±1.00 µmol/L; 192.00±2.00 µmol/L) across days 10, 20, and 30 respectively. In contrast, the lowest GSH level in the treated group, especially the scented candle group (71.25±48.75 µmol/L) on day 10 and the mixed exposure group (69.75±32.25 µmol/L) on day 30, suggesting that exposure to air freshener and scented candle products may reduce GSH levels over time. Statistically, rows, no significant differences (NS) were observed within each group over days, indicating that GSH levels remained relatively stable within each treatment across the study period. In contrast, comparison between groups (columns analysis) showed statically significant differences ($p \leq 0.05$) at each day (Table 5).

Table 5. Mean value±SE of GSH levels µmol/L in experimental animals exposed to air freshener spray, scented candle, mixed exposure and control group after10, 20, and 30 days

Group	Mean±SE of GSH µmol/L			L.S.D.
	10 day	20 day	30 day	
Air fresheners (A)	86.25±26.25 B a	93.50±3.50 B a	81.50±61.00 B a	33.61 NS
Scented candle (B)	71.25±48.75 B a	88.25±5.75 B a	88.75±8.75 B a	28.55 NS
Mix (D)	82.50±30.00 B a	83.75±43.75 B a	69.75±32.25 B a	28.06 NS
Control (C)	191.00±11.00 A a	183.00±1.00 A a	192.00±2.00 A a	37.21 NS
L.S.D.	27.34 *	31.08 *	26.95 *	---

Different capital letters in column and small letters in row indicate significant differences * ($p \leq 0.05$). NS: Non-significant.

The findings of the present study are consistent with those reported by Airaodion et al. (2020a), who assessed the impact of air freshener exposure on oxidative stress and immune function in male Wistar rats. Both studies demonstrated a notable reduction in serum GSH levels in the exposed groups compared to the control group. These observed similarities supports the hypothesis that prolonged inhalation of air fresheners impairs redox homoeostasis by exhausting GSH reserves.

Glutathione functions by detoxifying hydrogen peroxide and lipid peroxide through electrons donation, converting these reactive species into harmless byproducts such as water and oxygen. This process plays a critical role in shielding cellular components including lipids from oxidative degradation. The observed decrease in GSH levels among rats exposed to air freshener for 28 days may be attributed to oxidative stress induced by air pollutions, as well as the direct binding of GSH with reactive intermediates formed during pollutant oxidation (Airaodion et al., 2020d). These findings are consistent with the results of the present study.

The reduction in blood antioxidant levels observed after air freshener exposure is likely due to the presence of VOCs, which alters the equilibrium between ROS and the body's defense mechanisms, leading to oxidative stress as a result of excessive ROS generation (Yang et al., 2007). Disruption of the antioxidant defense system increases the body's susceptibility to various diseases (Wang et al., 2013). Chronic exposure to air pollutants, particularly elevated concentrations of particulate matter, has been linked to cancer development through mechanisms involving cellular oxidative stress (Cocârţă et al., 2021). Previous studies have reported that air fresheners induce oxidative stress by increasing ROS generation. The adverse effect of air freshener on male sex hormones could also result from increased free radicals production and oxidative stress induction by these products (Airaodion et al., 2020e).

4. CONCLUSION

The findings of this study reveal that exposure to air freshener and scented candle induced a clear state of oxidative stress in the treated rats, as evidenced by significant biochemical alterations. GSH levels decreased markedly in all treated groups compared to control, reaching the lowest value of 69.75 µmol/L in the 30 day mixed exposure group, versus 191.00 µmol/L in controls. Conversely, LPO levels increased across all exposure duration, with the highest value of 22.94 nmol/mL recorded in the mixed exposure group after 30 days. SOD levels progressively rose, reaching 5.54 ng/mL in the 30 days spray group compared to 3.16 ng/mL in controls. GPX levels also increased, with highest level of 1,745.48 pg/mL in 30 day spray group. Collectively, these results demonstrate that exposure disrupted redox balance and caused cellular injury, highlighting the pro-oxidant and potentially toxic nature of air freshener sprays and scented candles.

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AUTHOR CONTRIBUTIONS

Conceptualization, Al-Maliki MAS; Methodology, Al-Maliki MAS; Investigation, Al-Maliki MAS; Formal analysis, Al-Maliki MAS; Data curation, Al-Maliki MAS; Writing original draft, Al-Maliki MAS; Writing review and

editing, Al-Maliki MAS; Visualization, Al-Maliki MAS; Supervision, Al-Easawi NAF; Project administration, Al-Maliki MAS, Al-Easawi NAF.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that this study was conducted without any conflicts of interest.

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