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Effects of spray air fresheners and scented candle exposure on air quality, growth, and locomotor activity in rats

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ABSTRACT

Introduction: Air fresheners and scented candles release harmful chemicals indoors, potentially posing health risks with prolonged exposure.
Materials and methods: This study investigated the effects of inhaling emissions from these products on growth and locomotor activity in rats. Forty rats (180–200g) were randomly assigned to four groups: air freshener (A), scented candle (B), combined exposure (D), and control (C). Exposures were conducted in a controlled inhalation chamber for 10, 20, and 30 days (1 h/day), with 15 min of direct exposure. Environmental parameters (Particulate Matter ($PM_{2.5}$, PM_{10}), Total Volatile Organic Compounds (TVOCs), Formaldehyde (HCHO), temperature, and humidity) were monitored at three time intervals: 0–15 min (emission), 15–30 min (without emission), and 30–60 min (without emission), using a portable monitoring device.
Results: Significant increases ($P \leq 0.05$) in $PM_{2.5}$, PM_{10}, TVOC, and HCHO were observed in group D compared to other groups. Rats in group D showed reduced growth rate and locomotor activity.
Conclusion: These findings suggest that combined exposure worsens indoor air quality and may impair physiological and behavioral health.

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Introduction

Air Fresheners (AFs) are consumer products commonly used in various indoor environments to promote cleanliness and mask unwanted odors by emitting aromatic compounds. Recent studies, however, indicate that many of these products act as major indoor pollutants and may pose risks to human health [1, 2]. AF emits more than one hundred different chemical compounds. This includes Volatile Organic Compounds (VOCs) (terpenes such as limonene and beta-pinene; terpenoids such as linalool, as well as ethanol, formaldehyde, benzene, xylene, and others) and Semi-Volatile Organic Compounds (SVOCs) (such as phthalates) [3]. Numerous studies have highlighted the hazardous nature of VOCs, emphasizing their capacity to induce adverse health effects and contribute to indoor air pollution [4].

Synthetic fragrances used in scented candles often contain phthalates, a group of chemical compounds commonly added to enhance scent longevity. During the burning process, these phthalates are released into the air, where they can be inhaled or absorbed through the skin. Once they enter the bloodstream, phthalates may exacerbate allergic reactions and respiratory conditions such as asthma. Multiple studies have linked the use of scented candles and air fresheners with adverse health effects, including headaches, coughing, shortness of breath, skin irritation, and allergic rhinitis [5]. In addition, air fresheners directly emit a range of chemical compounds into indoor environments during use, which are considered primary pollutants, including VOCs such as terpenes. These primary emissions can subsequently undergo chemical reactions with indoor oxidants, particularly ozone, leading to the formation of secondary pollutants such as formaldehyde [6].

Air pollution occurs when the concentration of certain substances exceeds safe limits, potentially compromising human and animal comfort or causing harm to plants. These substances are referred to as air pollutants and may exist in particulate, liquid or gaseous forms [7]. VOCs are a major class of air pollutants; typically occur at considerably higher concentrations indoors than outdoors, and these compounds generate ozone or odors. All types of air fresheners, even those marketed as green and organic, have the potential to release VOCs that may be harmful [8, 9]. Several VOCs, including fragrant chemicals, are classified as toxic or hazardous substances under United States federal laws and each product was observed to release at least one of these chemical compounds. These products often serve as sources of compounds known to affect health in multiple ways, such as endocrine system disruption [10].

While it is well established that Particulate Matter (PM) is a widespread atmospheric pollutant; PM can act as an allergen; upon exposure, individuals may develop immune mediated allergic inflammation. This plays a role in promoting negative health effects and worsening indoor air pollution [11, 12]. Furthermore, burning scented candles also leads to the emission of PM; the fine PM released during scented candle burning is small enough to penetrate the respiratory system and accumulate in the alveolar of the lungs. Accumulation of such particles in the alveolar is potentially linked to various respiratory and cardiovascular health issues [13]. Exposure to fragrances may lead to severe adverse health outcomes, raising public concern, especially given their influence on perceived indoor air quality [14].

Experimental studies have shown that exposure to indoor air pollutants released from air fresheners may adversely affect normal growth in laboratory animals. Reduced weight gain has been attributed to disruptions in food intake efficiency and alterations in nutrient absorption and metabolism induced by fragrance related compounds [15]. In addition to growth related effects, exposure to indoor air pollutants has been associated with changes in locomotor activity, with evidence suggesting that behavioral responses may vary depending on the chemical composition of the pollutants and the duration of exposure [16].

The current study aims to evaluate the impact of exposure to air fresheners on indoor air quality by observing locomotor activity and growth rate in rats and assessing air pollutants such as Volatile Organic Compounds (VOCs), formaldehyde (HCHO) and other pollutants.

Materials and methods

Collection of air-freshener samples

For this study, two (2) air fresheners (non-combustible) and scented candle (combustible) samples were a convenience sample randomly selected from the air freshener sections of various stores from different manufacturers and sellers in Baghdad city. All two products are available and can be used by consumers. However, the same brands and product types are widely distributed both throughout Iraq and 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 the time of analysis. Main features of the investigated air fresheners are summarized in Table 1. Samples of air freshener, collected from October 2023 to December 2023. Fig. 1 shows the locally manufactured inhalation exposure unit and its components.

Table 1. Main characteristics of the investigated air freshener and scented candle samples

Sample type	Container material	Fragrance description	Country of manufacture	Volume \Weight
Air Freshener	Plastic Spray Can	Not specified (commercial formulation)	Turkiye	500 mL
Scented Candle	Glass Beaker	Twisted Peppermint (Cool Peppermint, Sugared Snow, Vanilla Buttercream)	USA	411g

The study was done with assistance of the animal house unit at Biotechnology Research Center\ University of Nahrain from January 2024 to June 2024.

The inhalation exposure unit

The inhalation exposure unit is a dynamic system, whole-body chamber most widely used in studies involving prolonged exposure and large numbers of test laboratory animal's subjects. Designed according to the WHO specifications which mentioned by [17].

The inhalation unit manufacturing

The laboratory unit that used in this study consists of the following parts, which appear in:

The inhalation exposure chamber: this part represents a 5 mm transparent Plexiglas chamber, manufactured locally with the following dimensions (50 cm length x 40 cm width x 40 cm height) with a capacity of 80 L. (1), the chamber has three openings (input, output and ventilation), input and output openings are on both sides of the chamber, while ventilation opening is a narrow strip along the top cover of the chamber. The chamber has been provided with a thermometer to determine temperature inside the chamber during the inhalation exposure experiment. The animal wastes fall on removable aluminum foil covered the chamber floor, to facilitate cleaning after each daily exposure.

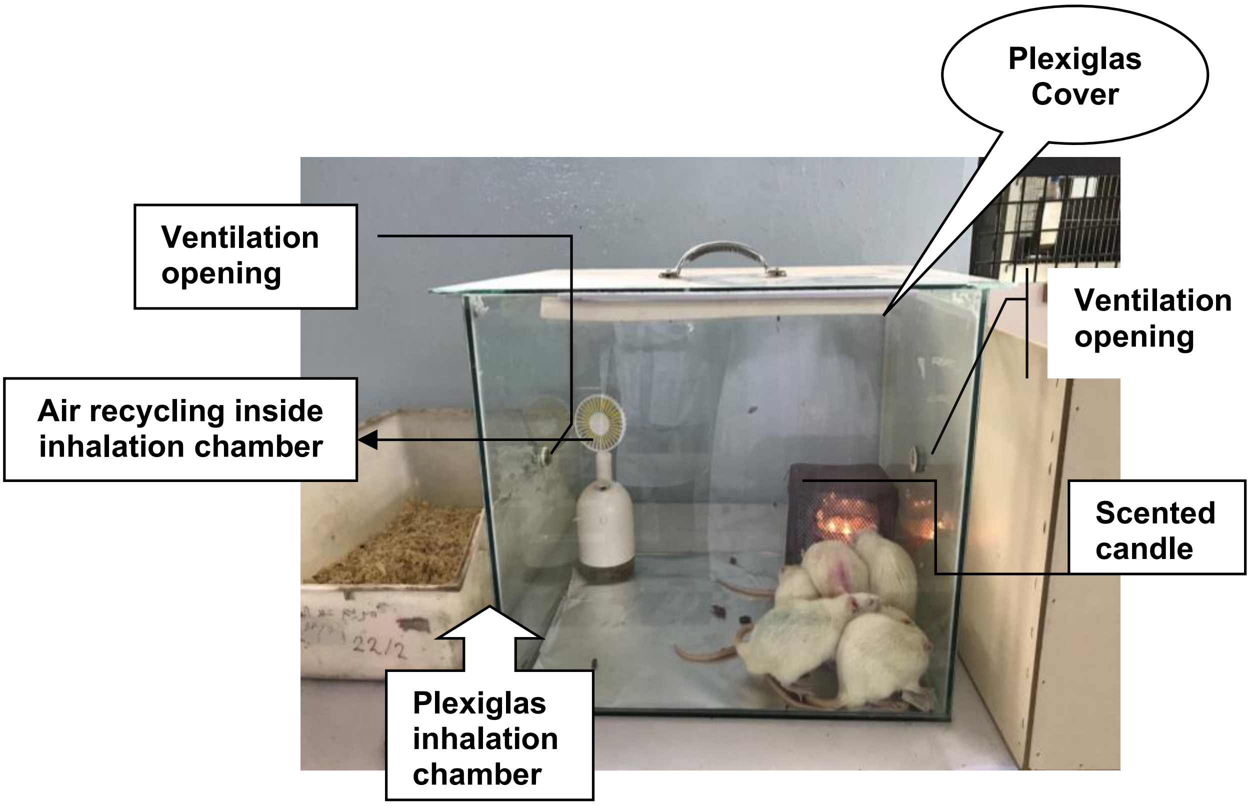

Fig. 1. The locally manufactured inhalation exposure unit and its components

Air samples collection device

A portable device was used to detect the concentrations of Particulate Matter ($PM_{2.5}$), Particulate Matter (PM_{10}), formaldehyde (HCHO), Total Volatile Organic Compound (TVOC), Temperature (T) and relative Humidity (RH). A sensor attached to the device to enable direct readings for air samples concentrations. The field digital device is produced by Temtop LKC-1000S+ Air Quality Detector/IAQ monitor.

Monitor overview

It is a laser particle multi-functional detector of model LKC-1000S+ (US) with Display mode: TFT color LCD screen. It can accurately detect indoor air quality. Adoption the high precision electrochemical formaldehyde and laser particle sensor. It can also directly convert the concentration of pollutants in the air into visual data and provide air quality information to help effectively protect health. The monitor operates within temperature range (0-50 °C) and relative humidity range (0-90 %) at atmospheric pressure condition: 1 atm. The monitor measurement index and range for $PM_{2.5}$ are of range (0-999 μg/m³) with resolution (0.1 μg/m³), for PM_{10} are of range (0-999 μg/m³) with resolution (0.1 μg/m³) and for formaldehyde is of range (0-5 mg/m³) with resolution (0.01 mg/m³).

Measurements of air samples concentration within inhalation exposure unit

The parameters that were detected by the monitor were TVOC (mg/m³), Formaldehyde (HCHO) (mg/m³), $PM_{2.5}$ and PM_{10} (μg/m³), Humidity (%), and Temperature (°F) and inside the inhalation exposure for three animal groups A, B and D, for comparison with control group (group C). For measurements concentrations, the device inserted inside the chamber. Concentration measurements taken at the start, middle and end of the exposure period. The one hour exposure period was divided into : start (0-15 min) during this time , group A was exposed to air freshener spray (2.5 mL) , group B exposed to scented candle burning , group D exposed to combination of both exposure (AF+SC) while group C was exposed to fresh air. While middle (15-30 min) and end (30-60 min) without air freshener spraying and burning candle for all groups (A, B and D).

Experimental animal groups

Forty (40) male Wistar rats, weighing between (180-200g), were used in this study. They were acclimated to laboratory conditions for seven days before the experiment began. Animals were purchased from the National Center for Drug Control and Research. After the acclimatization period, the rats were weighed, recorded and divided into four groups; each group included ten rats into exposed and control groups. The rats were kept in polypropylene cages (30cm X 15cm X 15cm) covered with wire grid covers and maintained at a temperature range of 27–29 °C. The cages were routinely cleaned using hot water and alcohol, followed by bedding with sawdust that was maintained in a dry state and changed from time to time. Cages were kept under standard animal house conditions where the humidity ranged between 56-58 % and 12±2 hrs (light/day). Rodent diet and drinking water were available ad libitum, except during inhalation exposure.

Experimental design

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

Group (A): 10 rats were exposed to inhalation air freshener spray (2.5 mL). The whole exposure duration was 60 min daily after opening the freshener for 15 min.

Group (B): 10 rats were exposed to inhalation of scented candle. The whole exposure duration was 60 min daily after opening scented candle for 15 min.

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

Group (D): 10 rats were exposed to inhalation mixture (air freshener spray (2.5 mL) and scented candle) at the same time. The exposure duration was 60 min daily after opening at (air freshener (liquid) and scented candle) at same time for 15 min.

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.

Opened Field apparatus for locomotors activity measurements

The goal of using this tool is to determine the locomotor activity of laboratory animals after exposure to the studied pollutants. The opened field apparatus, (Fig. 2), represents a square coverless white plywood box made locally; its dimensions are (72 cm x 72 cm x 36 cm). The box side's height is (36 cm), which is sufficient to prevent animals from escaping during the test. Straight lines are drawn on the box floor to divide it into sixteen (18 x 18 cm) even squares. These dimensions and design are recommended by Brown et al. [18].

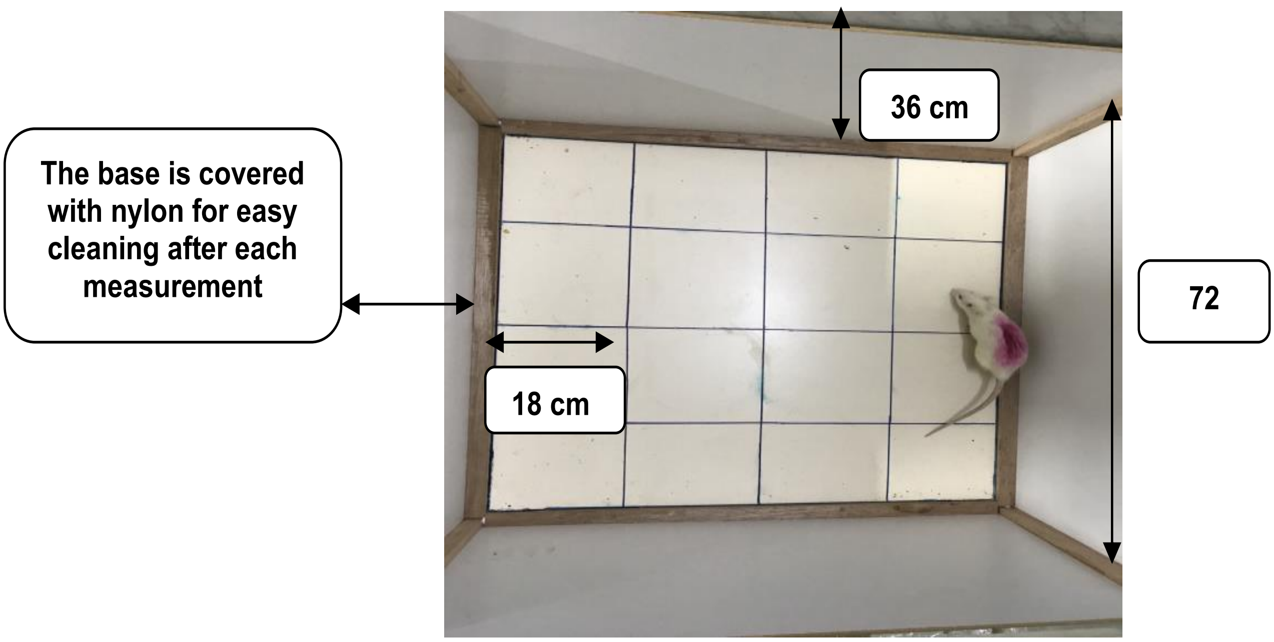

Fig. 2. The open field apparatus

Toxicity observations

The observations on toxicity involved the locomotor activity, and body weight changes.

Locomotors activity observations

To assess locomotor activity after exposure to air fresheners, animals were removed from the inhalation chamber and were placed in one of the four corners of the opened field apparatus facing the center and allowed to move on the floor of the opened field apparatus for 2 min according to the procedures recommended by Prut & Belzung [19]. The number of line crossings (Frequency with which the rat crossed one of the grid lines with all four paws) was used as a scored of total locomotors activity. This test was applied weekly for each exposed animal individually. locomotor activity testing was conducted in the morning during the light phase under natural laboratory lighting conditions. Illumination was kept constant throughout the testing period. The apparatus was cleaned with 70 percent ethyl alcohol and permitted to dry between each animal test to avoid odor cues as mentioned by Adjene and Abudu [20].

Animals' body weight changes

The body weight of animals was measured at the beginning of the research (before the inhalation exposure) and also regularly once a week until they was sacrificed and recorded separately for each experimental and control group. During non exposure periods, animals were housed in groups of three to five per cage. The growth rate per week was then calculated using the formula of Iranloye and Bolarinwa [21]: Growth rate per week = (weight at the last week- weight at the first week) / weight at the last week.

Every experimental procedure was 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).

Statistical analysis

The Statistical Package for the Social Sciences was utilized to evaluate the impact of different groups or factors on the study parameters. Differences among the experimental groups were analyzed using ANOVA. When significant differences were observed, comparisons between group means were conducted using the Least Significant Difference (LSD) post hoc test. Data are presented as mean ± standard error (SE), and statistical significance was set at $P \leq 0.05$ [22].

Results and discussion

Exposure chamber experiment

The temperature (°C) and relative humidity (%)

Regarding temperature, highest mean value (34.00±0.58 ºC) was recorded in the scented candle during start exposure, while the control group had a mean value of (29.07±0.56; 28.73±0.88; 29.17±0.43 ºC) during the start, middle and end exposure periods, respectively (Table 2). As shown in Table 2, within group comparisons revealed a statistically significant difference ($P \leq 0.05$) in group B compared with the other groups, with the observed difference exceeding the calculated LSD value (4.237).

Table 2. Mean value ± SE of temperature (°C), measured in inside air of examined chamber by air freshener and scented candle and mix

Group	Mean ±SE of T (°C)			L.S.D.
	Start	Middle	End	
Air fresheners (A)	28.30±0.85 B a	27.43±1.10 C a	28.03±0.09 B a	2.791 NS
Scented candle (B)	34.00±0.58 A a	33.67±2.02 A a	29.40±0.23 A b	4.237 *
Mix (D)	31.63±1.22 AB a	30.67±1.45 AB a	29.10±0.58 A a	3.973 NS
Control (C)	29.07 ±0.56 B a	28.73±0.88 BC a	29.17±0.43 A a	2.26 NS
L.S.D.	3.29 *	3.077 *	0.88 *	---

*Different capital letters in column and small letters in row indicate significant differences * (P≤0.05). NS: Non –significant.

Regarding relative humidity, lowest mean value (56.67±0.33; 54.00±0.58; 55.33±0.33%) was recorded in the scented candle during the start, middle and end exposure periods, respectively, while the control group had a mean value of (58.06±0.58; 58.13±0.46; 57.67±0.66 %) during start, middle and end exposure period, respectively. As shown in Table 3, within group comparisons revealed a statistically significant difference (P≤0.05) in group B compared with the other groups.

Table 3. Mean value ± SE of relative humidity (%), measured in inside air of examined chamber by air freshener and scented candle and mix

Group	Mean ±SE of RH (%)			L.S.D.
	Start	Middle	End	
Air fresheners (A)	57.67±0.66 A a	56.83±1.01 A a	57.33±0.33 A a	2.514 NS
Scented candle (B)	56.67±0.33 A a	54.00±0.58 B b	55.33±0.33 A ab	1.489 *
Mix (D)	57.67±0.33 A a	57.33±0.33 A a	56.00±1.52 A a	3.194 NS
Control (C)	58.06±0.58 A a	58.13±0.46 A a	57.67±0.66 A a	1.997 NS
L.S.D.	2.49 NS	2.71 *	2.567 *	---

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

In the current study, temperature and relative humidity were recorded at 0-60 min intervals inside the inhalation chamber during each experiment. Temperature and relative humidity were considered potential confounding factors in this study and were continuously monitored to account for concentrations and exposure outcomes.

The study has found that the highest mean temperature values were observed in the scented candle group during the start exposure compared with other groups according to Tables 2 and 3. On the other hand, relative humidity tends to decline when air temperature rises. This is because RH reflects the ratio between the actual water vapor present in the air and the amount needed to saturate it. As temperature increases, the air's capacity to hold moisture also expands, leading to a lower proportion of water vapor relative to saturation, and thus a decrease in RH [23]. TVOC and HCHO concentrations tended to increase as temperatures rose. Significant seasonal patterns of indoor pollutant concentrations have been observed in homes, mainly based on the relative strength of indoor and outdoor sources, room temperature and ventilation rates. Temperature had an impact on the rate at which pollutants are released from indoor materials. For instance, indoor concentrations for most pollutants were lower in winter season than in summer season in a study in Japan. Particularly, HCHO was strongly associated with temperature. According to a study conducted in Egypt; temperature and relative humidity were the main factors affecting indoor HCHO levels, with higher concentrations recorded in summer season. In general, indoor pollution sources typically have a higher effect on personal exposures during winter season, when indoor ventilation is poor and individuals remain indoors for extended periods [24,25]. Particulate matter and volatile organic compounds are impacted by relative humidity, temperature and also ventilation. As a result, indoor exposure levels can differ significantly based on the specific conditions in which air fresheners are used [26].

$PM_{2.5}$ and PM_{10}

The highest mean concentration of Particulate Matter ($PM_{2.5}$) was observed in air freshener and scented candle group (group D) at the middle exposure time (61.00±3.78 μg/m³), compared with the control group (8.00±0.58 μg/m³) during the start, middle and end times respectively (Table 2). According to the results mentioned in Table 4, there were statistically significant differences ($P \leq 0.05$) in all groups compared with the control were not significant differences, Table 4. When compared with the limits by the World Health Organization [27], which are less than (25 μg/m³) where all groups exceeded this limitation in the present study during start; middle and end exposure times; in comparison with the control group during all exposure times, while the air freshener group at the end exposure time was within acceptable limits.

Table 4. Mean value ± SE of $PM_{2.5}$ (μg/m³), measured in inside air of examined chamber by air freshener and scented candle and mix

Group	Mean ±SE of $PM_{2.5}$ (μg/m³)			L.S.D.
	Start	Middle	End	
Air fresheners (A)	31.00±4.943 B a	22.00±1.15 C ab	12.43±1.10 B b	10.36 *
Scented candle (B)	35.30±6.67 AB ab	45.30±0.90 B a	27.63±1.44 A b	13.77 *
Mix (D)	42.66±4.09 A b	61.00±3.78 A a	34.40±5.94 A b	16.28 *
Control (C)	8.00±0.58 C a	8.00±0.58 D a	8.00±0.58 B a	1.99 NS
L.S.D.	8.72 *	8.77 *	7.51 *	---

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

While PM_{10} levels, the present study results implied that the highest mean of PM_{10} (79.76±1.42 µg/m³) was scored in group D during the middle exposure time, while the lowest mean was scored in the control group during all exposure times, as it did not exceed (14.67±1.20 µg/m³) (Table 5). Statistically significant differences (P≤0.05) in PM_{10} concentrations were observed among all groups compared with the control group, as shown in Table 5. The comparison of PM_{10} determinants according to the WHO [27], which should be less than (50 µg/m³); It was unacceptable in the studied group B during start and middle exposure times; and group D during all exposure time; in comparison with the control and air freshener groups.

Table 5. Mean value ± SE of PM_{10} (µg/m³), measured in inside air of examined chamber by air freshener and scented candle and mix

Group	Mean ±SE of PM_{10} (µg/m³)			L.S.D.
	Start	Middle	End	
Air fresheners (A)	46.36±3.78 A a	31.97±2.53 C b	27.97±4.05 C b	12.182 *
Scented candle (B)	51.00±4.72 A a	62.67±2.60 B a	35.67±3.05 B b	12.388 *
Mix (D)	59.63±5.25 A b	79.76±1.42 A a	52.00±4.58 A b	14.209 *
Control (C)	14.67±1.20 B a	14.67±1.45 D a	14.46±2.15 D a	5.711 NS
L.S.D.	11.37 *	16.44 *	13.17 *	---

*Different capital letters in column and small letters in row indicate significant differences * (P≤0.05). NS: Non –significant.

According to Tables 4 and 5, $PM_{2.5}$ and PM_{10} concentrations decreased progressively over time, with the lowest values recorded at the end of the exposure period. The data represent one hour measurements averaged across all experimental groups. Higher $PM_{2.5}$ and PM_{10} levels were observed during the start of the exposure phase (0-15 min) following air freshener spraying and combined exposure. Concentrations increased again during the middle phase (15-30 min). Particularly in the scented candle group after extinguishing, and then declined during the final phase (30-60 min) compared with the control group.

Particulate Matter (PM) consists of fine particles containing heavy metals, black carbon, polycyclic aromatic hydrocarbons, which can penetrate deeply into the respiratory tract and cause adverse human health [28, 29].

With regard to spraying air freshener; the concentration of particles increased with higher spray intensity during (0-15) min. The particle number concentration showed a tendency to decline shortly after spraying; it further decreased beyond the end period; particularly after 30 min. This agrees with the work of a study [26]. Candle burning increases personal exposure to PM air pollution [30]. According to [31], several studies characterized indoor $PM_{2.5}$ associated with the use of scented candles. Two studies have reported $PM_{2.5}$ concentrations of 70 and 36 $\mu g/m^3$ during candle burning.

The findings of this study indicate that burning of a scented candle significantly impacts indoor air quality by elevating particulate matter levels. It has been noted that more particles are generated during the extinguishing period of scented candle than during the burning period, suggesting a significant influence of the soot mode. $PM_{2.5}$ and PM_{10} levels exhibited a significant decline over time across all groups, likely due to gravitational settling. Also the results of the present study agree with the results of a previous study done by many researchers [32]. According to [33] a regression analysis indicated that one percent duration spent in proximity to scented candle was related with eight percent increase in personal $PM_{2.5}$ exposure. This indicates that one hour of candle use would cause an increase of thirty three percent in personal $PM_{2.5}$ exposure. There is a possibility that emission of PM from burning scented candle can induce respiratory symptoms in people with sensitive airways.

Total volatile organic compound (TVOC)

Regarding TVOC, this study has found that air freshener has generated a mean of (1.446±0.27; 0.700±0.03; 0.500±0.10 mg\m^3); while the scented candle has given a mean of (1.20±0.24; 0.640±0.11; 0.483±0.15 mg\m^3), while group D has recorded a mean of (2.25±0.12; 1.84±0.08; 1.14±0.12 mg\m^3); while fresh air has recorded a mean of (0.067±0.01; 0.070±0.02; 0.070±0.02 mg\m^3) during start, middle and end exposure period, respectively (Table 6). According to Table 6, statistically significant differences ($P \leq 0.05$) were observed among the groups during all exposure periods (start, middle and end). At the start of exposure, group D showed a significantly different value compared with the other groups; particularly relative to the control group. The mean TVOC concentrations measured in the present study exceeded the limits of the World Health Organization (0.6 mg/m^3); in comparison with the control group [27].

Table 6. Mean value ± SE of TVOC (mg/m³), measured in inside air of examined chamber by air freshener, scented candle and mixture

Group	Mean ±SE of TVOC (mg/m³)			L.S.D.
	Start	Middle	End	
Air fresheners (A)	1.446±0.27 B a	0.700±0.03 B b	0.500±0.10 BC b	0.587 *
Scented candle (B)	1.20±0.24 B a	0.640±0.11 B ab	0.483±0.15 B b	0.618 *
Mix (D)	2.25±0.12 A a	1.84±0.08 A b	1.14±0.12 A c	0.392 *
Control (C)	0.067±0.01 C a	0.070±0.02 C a	0.070±0.02 AC a	0.052 NS
L.S.D.	0.711 *	0.536 *	0.525 *	---

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

At the instance when air freshener was sprayed, candle were burned and mixtures were used, TVOC concentration within the room drastically increased. As shown in Table 6 after about 15 min from exposure, TVOC concentrations approached an average low concentration and continue to decrease following. These results are in agreement with the study conducted by Jadach [34]. According to the study conducted by [26], the concentration of TVOC was found to be 10.6 percent to 57.2 percent higher when air freshener sprays were used. The vehicle's air management setting had an impact on the concentrations of both particles and TVOCs. According to [35], the VOC concentration rises following the use of air freshener and scented candles in an enclosed room. Based on the findings of a study by researchers [36], individuals may experience higher terpene exposure when burning scented candles indoors, particularly under conditions of inadequate ventilation. Poor ventilation allows terpene compounds to remain in indoor air for extended periods, resulting in continued inhalation exposure even after candle extinguishing. In addition, short term activities such as air freshener application or scented candle use can generate temporary high TVOC concentrations, contributing to elevated indoor TVOC levels during prolonged exposure to both continuous and intermittent sources [37]. Studies on the use of air freshener products could lead to an array of health related ailments as TVOCs have been found to be responsible for irritation of the lungs, nausea, headaches and other diseases until death. Susceptibility to lung cancer and other noncancerous ailments is commonly associated with people frequently exposed to TVOCs in the indoor environment [38].

However, it is somewhat challenging to compare the results obtained in the present study with those of other studies due to the specific characteristics of the materials used and the variability in both the concentration and qualitative composition of emitted TVOCs depending on their sources. In addition, differences in the types of instruments used and the underlying measurement principles among studies may further contribute to this variability.

Formaldehyde (HCHO)

Regarding HCHO, this study has found that air freshener have generated a mean of (0.480±0.19; 0.400±0.03; 0.193±0.05 mg\m^3); while scented candle has given a mean of (0.467±0.12; 0.400±0.06; 0.243±0.03 mg\m^3); while the group D has recorded a mean of (1.173±0.05; 1.066±0.08; 0.400±0.06 mg\m^3); while fresh air has recorded mean of (0.036±0.003; 0.030±0.01; 0.027± 0.01 mg\m^3) during start, middle and end exposure period, respectively. Within group comparisons showed no statistically significant differences between most groups, except for group D, which exhibited a significant difference at ($P \leq 0.05$). In contrast, comparisons among groups revealed significant differences at the start and middle exposure periods, while no significant difference was recorded at the end of the exposure period. According to WHO [27], the average concentration of formaldehyde should not exceed 0.08 mg\m^3. From Table 7, the average formaldehyde concentration exceeded the recommended criteria in all groups compared with the control group.

Table 7. Mean value ± SE of HCHO (mg/m³), measured in inside air of examined chamber by air freshener, scented candle and mixed exposure

Group	Mean ±SE of HCHO (mg/m³)			L.S.D.
	Start	Middle	End	
Air fresheners (A)	0.480±0.19 B a	0.400±0.03 A a	0.193±0.05 A a	0.412 NS
Scented candle (B)	0.467±0.12 B a	0.400±0.06 A b	0.243±0.03 A b	0.276 NS
Mix (D)	1.173±0.05 A ab	1.066±0.08 A a	0.400±0.06 A b	0.232 *
Control (C)	0.036±0.003 C a	0.030±0.01 B a	0.027±0.01 A a	0.0149 NS
L.S.D.	0.362*	0.592 *	0.417 NS	---

*Means having with the different big letters in same column and small letters in same row differed significantly. * ($P \leq 0.05$), NS: Non –significant.

HCHO is considered an indoor pollutant because of its known or suspected toxic, cancer-causing and gene-altering effects [39]. Formaldehyde (HCHO) has been implicated in initiating asthma and a range of allergy-related conditions. Findings from two studies revealed that exposure to elevated indoor formaldehyde concentrations is linked to a higher risk of developing asthma [40]. Using scented candles leads to serious health and environmental consequences, as they emit considerable amounts of specific indoor pollutants, particularly some light hydrocarbon species. Some scented candles released high amounts of toxic compounds, including formaldehyde, particularly during their burning. Consequently, improving our understanding of the risks linked to indoor pollution sources (including air freshener and scented candles) requires expanding the available data for better air quality management [41]. According to [42], the concentration of formaldehyde (HCHO) emitted from all tested air fresheners was generally below 10 μg/m³ immediately after use and gradually declined over time, indicating the role of ventilation systems in pollutant removal. In the case of scented candles, formaldehyde release appears to be primarily associated with the combustion process rather than the chemical composition of the candle itself. The use of air fresheners has been shown to generate both primary and secondary indoor pollutants, and emission levels can vary significantly depending on the product type. In some instances, formaldehyde concentrations remained constant or even increased following use.

Furthermore, long-term exposure to indoor air pollutants such as HCHO and TVOCs may result in a range of acute and chronic health effects, including sensory irritation, fatigue, headaches, and even carcinogenic outcomes. Notably, the indoor concentrations of both formaldehyde and TVOCs are typically two to ten times higher than those measured in outdoor environments [43].

Experimental animals testing

Growth rate

The highest mean value (0.227±0.01 %) of growth rate was recorded in the control sample after 10 days and lowest mean value (0.008±0.01 %) was found in the animal sample exposed to air freshener spray and scented candle group (D) after 30 days; Table 8. In fact all examined rats that were subjected to various air freshener sources had similar pattern of responses where they started at a lower growth rate and increased with passing the exposure periods but never exceeded that of the control sample after 30 days. Analysis of variance showed significantly different ($P \leq 0.05$) effects of both air freshener and scented candle sources and exposure periods upon the growth rate of body weight.

Table 8. Mean value ± SE of growth rate (%) variables measured in experimental animals exposed to air freshener and scented candle and mixed and control samples after 10, 20 and 30 days

Group	Mean ±SE of Growth rate (%) 10 day	20 day	30 day	L.S.D.
Air fresheners (A)	0.177±0.02 B a	0.094±0.01 B b	0.032±0.02 B c	0.056 *
Scented candle (B)	0.164±0.02 B a	0.084±0.02 B b	0.069±0.02 B b	0.062 *
Mix (D)	0.203±0.01 AB a	0.095±0.01 AB b	0.008±0.01 B c	0.066 *
Control (C)	0.227±0.01 A a	0.156±0.03 A a	0.178±0.03 A a	0.059 NS
L.S.D.	0.057 *	0.061 *	0.068 *	---

*Different capital letters in column and small letters in row indicate significant differences * (P≤0.05). NS: Non –significant.

Previous studies reported that animals exposed to air fresheners for a period of 28 days showed a reduction in body weight gain compared to the control group. In addition, changes in behavioral activity were observed, including decreased locomotor activity during the exposure period. These observations are consistent with the reductions in body weight gain recorded in the present study [44]. The reduced weight gain observed in exposed rats may be associated with metabolic alterations induced by inhalation exposure. Similar reductions in body weight following air freshener inhalation have been reported previously, where such effects were suggested to be related to altered energy utilization rather than normal growth [45].

In line with these results, Hur et al. [46] demonstrated that inhalation of essential oils could lower the efficiency with which food is converted into body mass, without necessarily affecting the total amount of food consumed. This supports the idea that air freshener exposure may interfere with normal metabolic function, contributing to reduced growth.

Locomotor activity

Regarding locomotor activity, the results of the current study have been found to range from the lowest mean value of (24.67±1.76 line /min) in group (D) sample after 30 days to the highest (53.67±0.88 line/min) in the control sample but after 10 days; Table 9. Comparisons among all groups across all exposure periods showed statistically significant differences (P≤0.05), as indicated by different big letters within the same column. The results demonstrate that exposure to air freshener and scented candle; especially the mixed exposure, significantly reduces locomotor activity compared with the control group. Furthermore, within group comparisons revealed that locomotor activity in the air freshener, scented candle and mixed exposure groups significantly decreased from day 10 to day 30 when compared with the control group.

Table 9. Mean value ± SE of locomotor activity(line\min) variables measured in experimental animals exposed to air freshener and scented candle and mixed and control samples after 10, 20 and 30 days

Group	Mean ±SE of Locomotor Activity (Line/min)			L.S.D.
	10 day	20 day	30 day	
Air fresheners (A)	42.67±1.45 B a	40.66±1.20 BC a	31.00±1.15 BC b	7.02 *
Scented candle (B)	42.67±1.20 B a	37.66±0.88 BC ab	33.00±1.53 B b	6.97 *
Mix (D)	40.33±0.88 B a	32.00±1.15 C bc	24.67±1.76 C c	7.37 *
Control (C)	53.67±0.88 A a	52.00±0.58 A a	52.00±1.15 A a	4.72 NS
L.S.D.	5.49 *	6.84 *	6.78 *	---

*Different capital letters in column and small letters in row indicate significant differences * (P≤0.05). NS: Non –significant.

Several studies have demonstrated that exposure to air fresheners can lead to behavioral changes in animals. For instance, many researchers [47] found that mice exposed to solid air fresheners exhibited a reduction in locomotor activity when assessed using the open field test. Similarly, our current findings revealed decreased movement activity in rats compared to the control group. In toxicological studies, [48] reported that the use of certain air freshener types was significantly associated with inflammatory responses, neurobehavioral disturbances, and hepatic cell damage in juvenile rats. Additionally, [49] investigated the combined effect of physical and psychological stress along with exposure to scented candle fumes on heart and lung health in mice. In their study, animals were exposed to candle emissions for 4.5 h daily, five days a week, over an eight-week period using an inhalation chamber. The results showed elevated levels of cardiac biomarkers such as hypoxia-inducible factor-1 alpha (HIF-1α) and Brain Natriuretic Peptide (BNP), suggesting that prolonged exposure to candle fumes under stress conditions may contribute to cardiopulmonary damage.

Conclusion

Air freshener spray and scented candle increase the concentrations of fine particles ($PM_{2.5}$ and PM_{10}), based on the air freshener experiment. This observation may be interpreted as evidence of particle growth, whereby volatile organic compounds emitted from air freshener spray and scented candle undergo secondary reactions in indoor air, producing oxidized vapors that can condense onto existing particles and contribute to particle formation and growth [50]. According to the examined parameters such as TVOC and HCHO concentrations inside the chamber has the most significant effect due to the air freshener spray and scented candle. The TVOC and HCHO concentration exceeded the permissible value during an exposure period of one hour. Finally, the present study indicates that air fresheners may adversely affect indoor air quality and could be associated with potential toxic effects under the studied exposure conditions, but also extends its impact on albino rats by causing toxicity in physical parameters such as decrease in body weight and loss of appetite. It also had an impact on locomotor activity by reducing movement in comparison with the control group.

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Competing interests

The authors confirm that there are no competing interest associated with this work.

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Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/ or falsification, double publication and/ or submission, redundancy, etc.) have been completely observed by the authors.

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