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Toxicity of environmental ozone exposure on mice olfactory bulbs, using Western blot technique

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ABSTRACT

Environmental ozone (O_3) exposure has adverse effects on different body systems. This experimental work aimed to study the effect(s) of O_3 exposure on the olfactory bulbs (OB) of Swiss Webster and C57BL/6J mouse strains, using Western blot technique. Both mice strains were exposed to different O_3 doses for different number of exposures and durations. The results indicated that O_3 exposure caused a significant increase in the level of the proteins involved in the oxidative stress state such as 4-hydroxynonenal (4HNE) and Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1), in addition to the total OB proteins in Swiss Webster mouse strain. However, this effect was not observed in C57BL/6J mouse strain. Furthermore, CYP1A1 was completely absent in the Green fluorescent protein (GFP) C57BL/6J O_3 exposed mice. Moreover, O_3 exposure caused a significant decrease in the body weight of the tested mice from the two strains.

1. Introduction

Air pollution is the presence of substances in the atmosphere in certain amount for a particular duration of time, which could be harmful to human life or could lead to particular changes in the weather and climate [1].

The olfactory system receptors are exposed directly to air pollutants making them more susceptive to the harmful effect of toxic substances causing damage to their neural tissue [2]. Furthermore, reviewed articles by Ajmani et al. [3] from 1950 to 2015 focused on human epidemiologic and pathophysiologic studies with some experimental studies on animals; showed a relationship between environmental air pollutant exposure and olfactory function. Moreover, these studies have explained how air pollutants enter the OB system.

The effects of oxidative stress on the olfactory bulbs have taken many researchers attention as a primary site for coding the odourous information. Therefore, any alteration in their components will lead to alteration in the stimulation process; resulting in changing of the olfactory and behavioural responses. Moreover, olfactory bulb is the main organ involved in memory-mindedness and perception of odours [4,5]. In addition, in animal models odour perception has an adaptive value for animal's survival. Furthermore, some authors found that the plasticity of the nervous system allows individuals to adapt themselves to any changes in the surrounding environment through memory and learning processes [6,7].

The increase of 4-hydroxynonenal (4HNE) level is considered as a biological marker for cellular toxicity due to the toxic effect of environmental O_3 exposure [8].

In addition, the role of CYPs genes has been reported as an indicator for tissue damage in mouse skin following O_3 exposure. Therefore, in this study, 4HNE and CYP1A1were chosen as indicators for the oxidative stress state which is produced due to O_3 exposure [9]. This experimental work aims to study the effect(s) of acute and chronic environmental ozone exposure on mice olfactory bulb from biochemical points of view by assessing the expression level of oxidative stress biomarkers (CYP1A1 and 4HNE) using immune Western blot technique and by measuring the level of plasma proteins. Also, this study aimed to observe the effect of O_3 exposure on the body weight of the tested mice.

2. Materials and methods

2.1. Ethical statement

Care and use of animals was conducted according to guidelines established by European Council Directives (609/1986 and 63/2010) and Italian laws (DL 116/92 and D. Lgsl. 26/2014) for the protection of animals used for scientific purposes. The experimental protocols were approved by the Committee for Animal Welfare of the University of

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Ferrara (OBA), by the Directorate-General for Animal Health of the Ministry of Health, and supervised by the Campus Veterinarian of Ferrara University.

2.2. Experimental animals

In this experimental study, two strains of transgenic mice line expressing the enhanced green florescent protein (e-GFP) protein were used. The first one was Swiss Webster which expresses the e-GFP protein under Calretinin (CR) promoter [10], while the second one wasC57BL/6 strain which expresses the GFP protein under the control of promoter tyrosine hydroxylase (TH) [11].

A total of 49 mice were used. Of whom; 27 were belonged to C57BL/6 J mouse strain and 22 were Swiss Webster mice. Of the 27 C57BL/6 J mic; 17 were the O_3 exposed group (ten WT and seven GFP), and 10 were the control group (not exposed to O_3). Moreover, of the 22 Swiss Webster mice; 15 were the O_3 exposed group while seven were the control group. Furthermore, the O_3 exposed groups of the two strains were further subdivided according to the exposure protocol.

The allocation of mice to O_3 exposed and control groups was performed randomly. The age of all of the mice was matched and it ranged between two to three months.

2.3. Experimental procedure

According to the study design, the body weight of all mice was measure (O_3 and non-O_3 exposed group). Then, the exposed group was further subdivided according to the exposure protocols.

Regarding acute O_3 exposure protocol, both strains were exposed to 0.5 ppm O_3 dose once for either 30 min or 1 h (h). While for chronic exposure protocol, Swiss Webster mice were exposed to 0.2 ppm O_3 dose for 2 h, two times for one day or 0.2 ppm O_3 dose for 2 h, two times for two days. On the other hand, C57BL/6 J mice were exposed to 0.8 ppm O_3 dose for 2 h, two times for successive five days.

Concerning chronic O_3 exposure protocol, first the tested mice body weight was measured. Then, the exposed mice were kept in a small cage 32 cm length, 13 cm width and 14 height, then were placed in a closed noiseless chamber with a diffuser connected to a variable-flux ozone generator. Ozone was produced from high-voltage current circulated in a tube; this tube contains aluminum chips and two electrodes that allow the conversion of oxygen into ozone. The O_3 concentration in chamber was adjusted according to the selected protocol and was continuously monitored by the O_3 sensor.

The first exposure started from 08:30 to 10:30 am. Following the first exposure, the body weight of the tested mice was measured, and the mice were retained to their original cage in the animal facility containing food and water, for two hours. At the end of the two hours, the body weight of the tested mice was measured again. Then, they were exposed to O_3 for another two hours. When the time was finished, the tested mice body weight was measured and the mice were retained to their home cage which contains food and water in the animal facility for the second day. Then, the same steps were repeated according to the days of the O_3 exposure protocol. In case of exposing C57BL/6 J mice, the steps were repeated for successive four days followed by single exposure on the fifth day. Whereas for acute O_3 exposure, the body weight of the O_3 exposed mice was measured before and after the exposure.

2.4. Samples

Following O_3 exposure, all of the tested mice were sacrificed and approximately 1 ml of blood was collected in 1.5 ml Eppendorf tube (Thermo fisher, Italy), containing 100 µl Na^+ citrate (Thermo fisher, Italy), centrifuged by LSE™ Compact Centrifuge (Thomas scientific, united states) at 2500 *g* at 4 °C for 15 min. Then the plasma was collected in new Eppendorf tube and stored at −20 °C. In addition, olfactory bulbs were collected and stored at −80 °C or freezed directly in liquid nitrogen (Thermo scientific, Italy) at −190 °C for homogenization. Then, 150 µl of lysis buffer (Thermo fisher, Italy), was added to the homogenized olfactory bulb samples, centrifuged at 3500 *g* at 4 °C for 15 min. Moreover, the supernatant liquid was collected in new Eppendorf tube and stored at −20 °C.

2.5. Bradford protein assay

It was used to measure the protein concentrations in plasma and homogenized olfactory bulb samples.

Briefly, each tested sample was diluted to appropriate dilution. Then, from each diluted sample a volume of 15 µl was taken and was placed in three wells on microtiter plate (Bio Rad) (5 µl for each single well). Also, 5 µl from each protein standard in a concentration of (0, 0.4, 0.6, 0.8, 1, 1.2,2, and 4 µg) was placed in separate well of microtiter plate. After that, 200 µl of 1X Coomassie Brilliant Blue (Biorad, Italy), was added to the diluted sample and protein standard wells. Following that step, the microtiter plate was incubated for 15 min at room temperature in the laboratory shaker, (Benchmark, Brasov, Romania) at 90 RPM speed.

Moreover, by using the spectrophotometer (Thermo Scientific, U.S) the absorbance of the known standard protein samples concentration and of the olfactory bulb and plasma samples (unknown protein concentration) was read at 595 nm.

Then, the standard curve was created by plotting the absorbance of Bovine Serum Albumin (BSA) (Biorad, Italy) standard values (y-axis) *versus* their concentration in µg/µl (x-axis).

For knowing the concentration of unknown total proteins in each sample (OB and plasma) using the created standard curve of BSA, the following steps were followed: first the mean of each three absorbance values of each single sample was calculated, after that from the curve formula the diluted concentration values were obtained then were multiplied by the appropriated dilution factor for each single sample (OB and plasma).

2.6. Western Blot (WB) technique

By using this technique, 4HNE in plasma and/or OB samples, and CYP1A1 in OB samples were measured in O_3 and non-O_3 exposed mice.

The total amount of proteins from OB and plasma was determined as 30 ul from each sample to be used for evaluating the level of 4HNE and CYP1A1.

The following antibodies were used to detect the oxidative stress biomarkers:

4HNE antibody (Biorad, Italy1:1000 / 6 ml).

CYP1A1 antibody (Thermo fisher, Italy1:1000–6 ml).

B-actin antibody (Biorad laboratory, Italy1:50000 − 10 ml).

Anti-Goat antibody used as secondary antibody for4HNE antibody.

Anti-Rabbit antibody (Biorad, Italy1:10000 − 10 ml) used as secondary antibody for CYP1A1 antibody.

Then the technique was performed according to the Bio Rad manufacture instructions.

2.7. Data analysis

Concerning Western blots results, Image studio lite software was used to measure the level of the tested biomarkers. Whereas, paired, unpaired t-tests and one- way ANOVA were used for the statistical analysis testing, using Graph Pad Prism (version 6) software.

The results of Western blots for each O_3 exposed group were compared with the related control group. While, for the effect of O_3 exposure on the body weight, the mean of the body weight for each single O_3 exposed mice was calculated from the measures of the body weight before and after each single exposure. Then, the values of the obtained means were compared together.

Table 1
Effect of 0.2 ppm/2 h/2times/1day O_3 dose on Swiss Webster mice.

Mice	Mice gender	OB 4HNE level (A.U) *		OB proteins level (A.U) *		Decrease in B.W % *
		Mean	SD	Mean	SD	
Control	5 Males + 2 Females	100	24.6	100	23.3	–
O_3 exposed mice	3 Females	172.3	30.1	184.7	39.1	Yes (5.1)

3. Results

This study provides the first report on the effect of acute and chronic environmental O_3 exposure on mice olfactory bulb using immune Western blot technique.

3.1. Swiss webster mouse strain

In this study, we aimed to measure the level of 4HNE in OB and/or plasma, CYP1A1 in OB, plasma protein in Swiss Webster and C57BL/6 J mouse strain following acute and chronic O_3 exposure doses compared to non-O_3 exposed mice. Also, we aimed to observe the effect of O_3 exposure on the body weight of the tested mice.

As shown in Tables 1–3 Fig. 1, the results of the immune Western blot technique (expressed as mean and standard deviation (SD)) showed that acute and chronic O_3 exposure caused a significant increase in the OB 4HNE, OB CYP1A1plasma 4HNE and plasma proteins in the O_3 exposed mice compared to the non-O_3 exposed group. However, mice exposed to 0.5 ppm O_3 dose for 30 min once did not show a significant increase in the level of OB 4HNE compared to the control group, Table 2.

3.2. C57BL/6J mouse strain

The effect of ozone exposure was evaluated in C57BL/6 J mouse strain to observe the effect of O_3 exposure among different mice stains.

Mice exposed to 0.8 ppm O_3 dose were divided by their genotype (WT and GFP), and both of them were exposed two times for two hours in four successive days followed by single exposure on the fifth day.

As shown in Tables 4–7, neither acute nor chronic O_3 exposure doses caused a significant increase in the level of 4HNE (OB and plasma), and plasma proteins in both phenotypes. Nevertheless, WT mice exposed to 0.8 ppm O_3 dose for two hours, two times for five days, showed a significant increase in the level of OB 4HNE compared with the control group, Table 6.

Moreover, the difference in the level of CYP1A1 between WT O_3 exposed mice and control mice (WT) was statistically non-significant and it was completely absent in the GFP O_3 exposed mice, Tables 4–7.

Moreover, O_3 exposure caused a significant decrease in the all of the O_3exposed mice regardless their strain and phenotype, Tables 1–7 and Fig. 1D.

In addition, the body weight of all of the O_3 exposed mice of the two strains was measured before and after each single exposure. Then, the mean was calculated for each single exposure values. As shown in Tables 1–3, 6–7 and Fig. 1D, the statistical analysis result test showed a significant decrease in the body weight of all of the O_3 exposed mice regardless their strain and phenotype, Tables 1–7 and Fig. 1D.

4. Discussion

In this study, two different mouse strains were used based on the results of other researchers, who studied the effects of O_3 exposure on mice and human subjects. Their findings have addressed a significant differences in susceptibility of environmental O_3 exposure among different mouse strains [12]. Furthermore, this susceptibility could be due to the differences in mice genetics [13]. Also, they found that, the genetic variability plays an important role in the predisposition of the environmental diseases. In addition, studies on human and animal models revealed that O_3 toxicity is species and strain dependent [14–17].

For example, a study by Savov et al. [16] showed differences in O_3 susceptibility to lung injury; their results indicated that C3H/HeJ and A/J mice strain were resistant to O_3 while, C57BL/6J and 129/SvIm O_3 were susceptible strains. Another study by Slade et al. [18] showed a significant difference in susceptibility to O_3 exposure in mice; the C3H/HEJ (C3) mice was reported to be less sensitive than C57BL/6J (B6) mice when exposed to the same O_3 dose.

4.1. Swiss webster mouse strain

Regarding Swiss Webster mice, the biochemical experiments revealed that O_3 caused an increase in the level of the oxidative stress biomarkers 4HNE (in OB and/or plasma samples) and CYP1A1 (in OB samples). Also, the level of OB proteins was elevated.

Our findings concerning the increase in the 4HNE and total proteins levels following O_3exposure support what have been published by other researchers. Connor et al. [19] exposed TLR4 mutant C3H/HeJ mice to 0.8 ppm O_3 dose for eight hours to study the effects of O_3 exposure on the lung. He found an increase in the 4-hydroxynonenal modified protein in the O_3 exposed mice compared to the control mice. In addition, another study in human bronchoalveolar lavage cells conducted by Hamilton et al. [20] demonstrated the role of 4HNE in the toxicity of human lung cells after O_3 exposure. Furthermore, the results obtained by Fakhrzadeh et al. [21] showed a significant increase in bronchoalveolar lavage fluid protein, lung macrophages and 4-hydroxyalkenals in the lung of 0.8 ppm O_3exposed wild-type mice for three hours. Also, another study by Kirichenko et al. [22] demonstrate that HNE is formed *in vivo* in C3H/HeJ mice following exposure to 2.0 ppm and 0.25 ppm O_3 doses for three hours. Furthermore, *in vivo* experiment in rats, conducted by Valentini et al. [23] who aimed to study the toxic effect of TiO_2 NPs using immunohistochemical technique. Their results indicated an increase in the level of 4-Hydroxynonenal due to lipid peroxidation in neuronal cells of hippocampus and cerebellum. Also, Bigagli et al. [24] have reported that, 4-hydroxynonenal and carbonyl residues are the products of protein oxidation; and their level has been increased *in vitro* model of cellular senescence.

Moreover, regarding the increase of CYP1A1 level following O_3 exposure, we obtained similar result to two studies on human epidermal keratinocytes exposed to 0.3 ppm O_3 dose, by Afaq et al. [25] and Zaid et al. [26]. Their results showed an increase in protein and mRNA expression of CYP1A1, CYP1A2, and CYP1B1 isoforms.

Based on the above mentioned results, it has been observed that O_3

Table 2
Effect of 0.5 ppm/30 min/once O_3 dose on Swiss Webster mice.

Mice	Mice gender	OB 4HNE level (A.U)		OB CYP1A1 level(A.U)*		OB proteins level(A.U)*		Decrease in B.W % *
		Mean	SD	Mean	SD	Mean	SD	
Control	5 Males + 2 Females	100	13.7	100	25.7	100	15.9	–
O_3 exposed mice	2 Females	111.5	21.2	221.6	43.7	146.5	23.1	Yes (1.6)

Table 3
Effect of 0.5 ppm/60 min/once O_3 dose on Swiss Webster mice.

Mice	Mice No./ gender	OB 4HNE level(A.U) *		OB CYP1A1 level (A.U)*		OB proteins level(A.U) *		Decrease in B.W % *
		Mean	SD	Mean	SD	Mean	SD	
Control	5 Males + 2 Females	100	11.9	100	18.78	100	16.3	–
O_3 exposed mice	3 Females	148.8	19.1	148.8	24.4	223.4	39.7	Yes (3.3)

*: Indicates $P < 0.05$.
SD: Standard Deviation.
B.W: Body Weight.
OB: Olfactory bulb.
CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1.
No.: Number.
A.U: Arbitrary Unit.
ppm: parts per million.
h: hour.
min: minute.
O_3: ozone.
4HNE: 4-hydroxynonenal.
%: percentage.
Control: non-O_3 exposed.

exposure has an adverse effect on mice OB. Which was proved by comparing the levels of oxidative stress biomarkers (4HNE and CYP1A1), plasma proteins between non-O_3 and O_3 exposed mice, in addition to the marked decrease in body weight following O_3 exposure.

From our biochemical results concerning Swiss Webster mice, we could suggest an alteration in the OB cells due to the generated oxidative stress state following O_3exposure.This assumption is supported by Colín-Barenque et al. [27] findings. Following O_3 exposure, he found an alteration in rates OB mainly in the granular layer.

4.2. C57BL/6J mouse strain

Our findings concerning the biological harmful effect(s) of different O_3 doses on C57BL/6 J mice did not match with what have been published by other researchers` work.

For example: Watkinson et al. [28] exposed C57BL/6J (B6) and C3H/HeJ (C3) mice to 2 ppm O_3 dose for 2 h to study O_3 toxicity in mice. Both strains showed a significant change in the level of O_3 toxicity biomarkers. Also, Kleebergeret et al. [29] used less O_3 doses (0.12 and 0.30 ppm for 72 h) than we used, to study the susceptibility of mice to

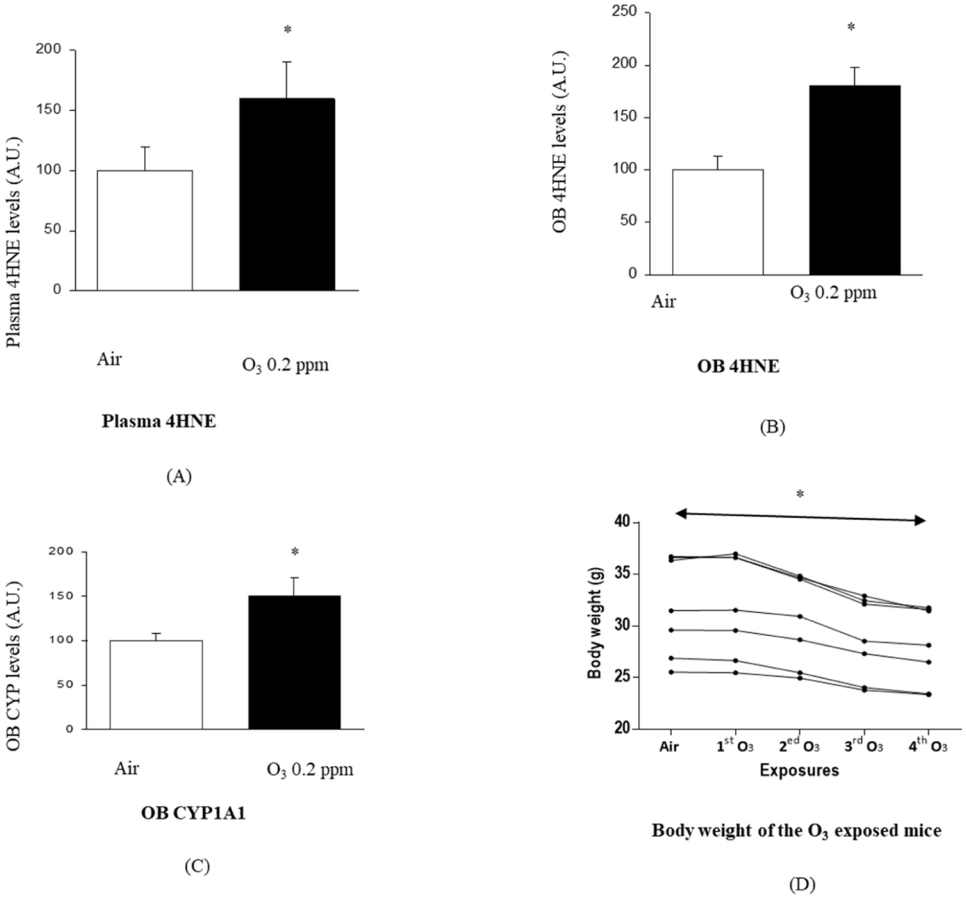

Fig. 1. Effect of 0.2 ppm O_3 dose for 2 h/2times /2days on Swiss Webster mice. (A): Plasma 4HNE level, (B): OB 4HNE level, (C): OB CYP1A1 level in Air (non-O_3 exposed) and 0.2 ppm O_3 exposed mice. (D): Decrease in the body weight of the O_3- exposed mice following O_3 exposure, (% of decrease in B W = 11.3).
Air: control (non-O_3 exposed group).
A.U: Arbitrary Unit.
*: Indicates $P < 0.05$.
OB: Olfactory bulb.
CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1.
g: gram.
%: percentage.

Table 4
Effect of 0.5 ppm/30 min/once on C57BL/6 J mice.

Mice No./ genotype	Plasma 4HNE level (A.U)		OB 4HNE level (A.U)		OB CYP1A1 level (A.U)		OB proteins level (A.U)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control 7 (WT)	100	25.2	100	13.5	100	14.5	100	16.7
O_3 exposed 2 (WT)	96.1	31.1	106.3	24.3	113.6	29.3	89.1	11.6

Table 5
Effect of 0.5 ppm/60 min/once on C57BL/6 J mice.

Mice No./ genotype	Plasma 4HNE level (A.U)		OB 4HNE level (A.U)		OB CYP1A1 level (A.U)		OB proteins level (A.U)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control 7 (WT)	100	19.6	100	13.4	100	29.8	100	16.5
O_3 exposed 3 (WT)	115.3	23.7	118.1	20.9	109.6	24.6	108.5	31.8

ozone-induced inflammation. He found a significant increase in the inflammatory cells after O_3 exposure.

Moreover, some *in vivo* studies tested the effect of different O_3 doses on C57BL/6 J mice.

Pulfer et al. [30] exposed the same strain to varying O_3 concentrations (0.5–3.0 ppm) for three hours to study the effect of O_3 exposure on the lung toxicity. His results indicated that β-epoxideand 6-oxo-3,5-diol have a role in O_3 toxicity in the lung of the exposed mice.

On the other hand, the immune Western blot results showed a complete absence of CYP1A1 in the OB samples of GFP O_3 exposed mice, although, it was detected in the $O_3$and control groups WT mice. This finding needs further investigation by using more specific techniques.

Furthermore, in our results we observed a significant decrease in the body weight of the O_3exposed mice regardless their strain and sex. Therefore, we assumed this finding to the effect of O_3 exposure on the peroxidation level of lipids. This presumption is supported by several published experimental findings. As Rivas-Arancibia et al. [31–34] and Pereyra-Muñoz et al. [35] correlated the increase in lipid peroxidation level to the generated oxidative stress state following O_3 exposure.

Moreover, comparing the exposure results of mice exposed to 0.5 ppm for 30 and 60 min from the two strains, the result indicates that C57BL/6J mice is more resistant to the harmful effects of environmental O_3 exposure compared with Swiss Webster mice strain, regardless the number of the tested mice of the two groups. Therefore, the differences in susceptibility to environmental O_3 exposure could be attributed to the differences in genetic background between the two strains. This finding matches what has been published by several researchers. For example: a study by Watkinson et al. [28] who aimed to study the differences in O_3 toxicity in C3H/HeJ (C3) and C57BL/6 J (B6) mouse strains. Their findings indicated that, both strains showed a significant difference in the response to environmental O_3 exposure, however, following 22 h of O_3 exposure B6 mice strain showed a significant increase in BAL fluid protein and cells, however, the number of neutrophils was significantly decreased in comparison with C3 mice strains. Moreover, they assessed the hypothermic response of both strains following O_3 exposure, they found that B6 mice were less dynamic than C3 mice strain. Moreover, they observed difference in the relationship between BAL parameters and the induced hypothermic response among the two strains following O_3 exposure. In addition, Slade et al. [18] have reported that, the differences in O_3 susceptibility among different mice strains could be due to variances in the delivered O_3 dose to the lung, which may be correlated to differences in the ability of the mice to lower their body core temperatures following O_3 exposure. Furthermore, Kleeberger et al. [29] observed differences in susceptibility to the airway inflammatory responses to subacute O_3 exposure (0.12 ppm and 0.30 ppm for 72 h) in B6 and C3 mouse strains. In their study, they found that 0.12 ppm O_3 dose caused a significant influx of polymorphonuclear leukocytes (PMNs), lymphocytes, alveolar macrophages and in the total BAL proteins level in both strains. On the other hand, 0.30 ppm O_3 dose showed a significant greater inflammatory response; in which B6 mice had high level of BAL proteins and great number of inflammatory cells in comparison to C3 mice strain. Moreover, Another study by Kleeberger et al. [36] was conducted to examined O_3-induced airway inflammatory responses using different inbred mouse strains which were exposed to 2 ppm O_3 dose for 3 h. Their findings indicated that, the magnitude of the inflammatory responses to O_3 was statistically different between B6 and C3 mice strain. Regarding the number of PMNs, the difference was 22-fold after 2 h of O_3 exposure and six-fold after 6 h following O_3 exposure. While for BAL proteins level, the difference was observed after 6 h of O_3 exposure and 24 h after O_3 exposure between the two strains.

In addition, the characterization of genetic mechanisms of responses to environmental pollutants could help in identification of susceptibility of individuals to these agents. This was much more clarified by Steven [37], in his study he used nine inbred mouse strains, including: 129/ J, A/ J, AKR/ J, BALB/ cJ, C3H/ HeJ, C57BL/6J, DBA/2J, SJL/J, and SWR/J (male, 5–6 wk) to determine if there is a significant difference in genetic contribution in susceptibility to lung injury and inflammation following exposure to single and repeated exposure to nitrogen dioxide (NO_2). His results showed intra-strain variations in epithelial injury, cellular inflammation (polymorphonuclear, epithelial cells, leukocytes and macrophages), lung injury and proteins level due to NO_2 exposure. This variation in the response to NO_2 indicated a great contribution of genetic background of mice to this agent. Also, he aimed to test whether the susceptibility to NO_2 and O_3 exposure between B6 and C3 was statistically different or not. The result indicated a significant difference in the response to these two pollutants among the two strains. Moreover, depending on all of his findings, he suggested that, the genetic susceptibility to NO_2 is significantly different from O_3 among all of the tested mouse strains. Furthermore, published epidemiological studies and clinical data on human showed marked difference in respiratory effect due to O_3 exposure among healthy and asthmatic individuals [38–41]. The difference in susceptibility to O_3 harmful effect was considered to be due to variation of human genetics [13].

5. Conclusion

In conclusion, Swiss Webster mouse strain is more sensitive to the

Table 6
Effect of 0.8 ppm/2 h/2times/5days on C57BL/6 J mice.

Mice No./genotype	Plasma 4HNE level(A.U)		OB 4HNE level(A.U) *		OB CYP1A1 level(A.U)		OB proteins level(A.U)		Decrease in B.W % *
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Control 8 (WT)	100	22.0	100	15.3	100	48.4	100	26.6	–
O_3 exposed 4 (WT)	144.4	41.1	165.3	28.4	112.3	36.8	110.9	23.6	Yes (27.8)

Table 7
Effect of 0.8 ppm/2 h/2times/5days on C57BL/6 J mice.

Mice No./genotype	Plasma 4HNE level(A.U)		OB 4HNE level(A.U)		OB CYP1A1 level(A.U)		OB proteins level(A.U)		Decrease in B.W % *
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Control 8 (WT)	100	18.1	100	32.1	100	48.4	100	42.0	–
7 (GFP)	112.3	22.4	105.1	38.6	Abs.	Abs.	108.3	32.8	Yes (24.1)

*: Indicates $P < 0.05$.
SD: Standard deviation.
B.W: body weight.
OB: olfactory bulb.
CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1.
WT: Wild Type.
No.: Number.
GFP: Green Florescent Protein.
Abs.: Absent.
ppm: parts per million.
h: hour.
min: minute.
O_3: ozone.
4HNE: 4-hydroxynonenal.
%: percentage.
Control: non-O_3 exposed.

harmful effects due to O_3 exposure than C57BL/6 J mouse strain. In addition, ozone exposure caused an increase in the level of the proteins involved in the oxidative stress state in the OB of Swiss Webster mouse strain. However, in C57BL/6 J mouse strain, this effect was not observed. Moreover, all of the O_3 exposed mice showed a significant decrease in the body weight following the exposure. Furthermore, the expression level of CYP1A1 following O_3 is affected by mice genotype.

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Author contribution

The corresponding author Samah Abd-Elrahim Batran has performed the followings: Designed the study, did the mice breeding, exposed mice to O_3, prepared the samples, performed the Bradford and Western blot techniques, performed the statistical analysis, did the literature search, performed the statistical analysis, interpreted the results and wrote the manuscript.

Declaration of Competing Interest

The author has no any conflict of interest.

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