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Investigation of Safety of Low-Dose Inhaled Chlorine Dioxide Gas on Pregnant Rats

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ABSTRACT

Background: Chlorine dioxide gas (ClO_2) has potent antimicrobial activity at low concentrations and plays an important role in infection control. This study investigates the effect of long-term inhalation of low-concentration ClO_2 by pregnant rats, followed by a two-week observational period for the offspring.

Method: Twenty-two pregnant Wistar-Albino rats were used in this study. exposed to ClO_2 during gestation ClO_2 released at 0.03 ppm for 24 hours/day from the AirDoctor® device was tested on 11 rats of them during the whole gestation period under stable conditions. The other 11 control pregnant rats were exposed to room air only. Body weight was recorded at baseline and day-18 of gestation. ALT and Creatinine serum levels were measured. Nasal cavity swabs were taken to test for bacterial microbiota at baseline and after 18 days of exposure to the gas. Offspring survival, weights, and teratogenic features were monitored for two weeks after delivery.

Results: No ClO_2-related toxicity signs were observed during the whole study period. No significant differences were observed either in body weights, hepatic and renal markers, microbiota ratios, or the number and weight of pups. No negative observations were recorded about the general health of the offspring.

Conclusion: Exposure of pregnant rats to 0.03 ppm ClO_2 for 24 hours/day from the AirDoctor® device by inhalation might have no observed toxic effects.

Introduction

Chlorine dioxide (ClO_2) is an inorganic chemical compound that exists as a yellow to reddish-yellow gas with an unpleasant odor and one unpaired electron in its molecular orbital (1,2). Generally, ClO_2 is obtained by the chemical reaction of sodium chlorite ($NaClO_2$) with an acid (3), according to such equation(4).

(1) $Cl_2 + H_2O \longrightarrow HOCl + HCl$

(2) $2NaClO_2 + HOCl + HCl \longrightarrow 2ClO_2 + H_2O + 2NaCl$

Net:

$2NaClO_2 + Cl_2 \longrightarrow 2ClO_2 + 2NaCl$

In many cases, $NaClO_2$ is sold as part of a kit and with an acid "activator" (5), for example, AirDoctor® Figure 1.

Figure 1. AirDoctor® device.

AirDoctor® contains $NaClO_2$ and natural inorganic substances (natural zeolite), and with acidity, the mixture turns into ClO_2. ClO_2 is currently approved by the United States Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) for many antimicrobial uses (6). It is considered a useful gas to be employed to prevent the transmission of respiratory infections in public places such as offices, schools, theatres, hospitals, and airports (2). The low concentration of ClO_2 is considered to be sufficiently effective against aerosol infection for the causative microorganism of nosocomial infection (7). The high concentration, it effectively inhibits microbial and viral activity (3).

Studies demonstrated that ClO_2 at low concentrations is one of the most effective biocides against microbes such as Gram-positive and Gram-negative bacteria, and enveloped and non-enveloped viruses. Moreover, some antifungal activities have been reported. However, the concentrations of ClO_2 used in such study reports were high (250– 3500 ppm). The antiviral activity of the low-concentration ClO_2 was mainly against enveloped viruses than non-enveloped viruses. For the bacteria, the ClO_2 solution was more effective against *Escherichia coli* (Gram-negative) than *Staphylococcus aureus (Gram-positive)*. Furthermore, ClO_2 would be useful for reducing the risk of *methicillin-resistant Staphylococcus Aureus* (MRSA) and swine-origin Flu-A infections in wet environments such as kitchens and bathrooms (7). It was also found that the ClO_2 at 0.03 ppm concentration is useful against mosquito-related infective diseases, such as malaria and dengue fever (8). Prior studies have demonstrated the effects of chlorine dioxide as a topical agent by killing bacteria, viruses, and fungi in less than one minute (9). Chlorine dioxide's antiviral activity is strong (10). In a recent study, the mechanism of chlorine dioxide's antiviral activity was explained by working on stopping the synthesis of proteins in the cell wall of the pathogen. Because it is a selective oxidizer, its mode of action is very similar to phagocytosis (11).

Moreover, chlorine dioxide can combat viruses through the process of selective oxidation through the denaturation of capsid proteins and subsequent oxidation of the virus's genetic material, rendering it

inactive. As there is no possible adaptation of the virus to the oxidation process, it can't develop resistance to the oxidation of chlorine dioxide, which makes it a promising treatment for any virus strain (11).

As an antibacterial, the oxidative potential of chlorine dioxide also attacks the lipids in the bacterial cell membrane, which will consequently increase its permeability and cause cell death and sterilization (2). In fact, chlorine dioxide assists the immune system in the body to destroy pathogens by providing it with activated oxygen (1).

Due to COVID-19 pandemic; the exposure of people to ClO_2 has increased. ClO_2-releasing kits were promoted for protection against infection with SARS-COV-2 virus (12). The proposal of using ClO_2 to prevent or treat COVID-19 was preceded by suggestions and claims for its use as a cure for other infectious diseases such as malaria and HIV, despite the lack of evidence of efficacy against that virus specifically (12,13). Interestingly, the SARS-COV-2 virus protein contains 54 tyrosine, 12 tryptophan, and 40 cysteine residues; all of these residues can react with ClO_2 leading to virus deactivation (14).

Previous studies had assessed the effects of ClO_2 on rat models at 100 ppm concentration. There were no signs of fetal malformations following ingestion, and no observed local or systemic side effects following topical application were detected (9). Besides, delay in brain development has been seen in animals as one of the toxicity effects when exposed to high levels of ClO_2 in animals before delivery and during early development after delivery (15).

The microbiota that is found in the upper respiratory tract are called gatekeepers to respiratory health because they prevent potential pathogens from overgrowing and disseminating toward the lungs. These microbiotas are strongly affected by certain circumstances such as the type of gases inhaled and can be pathogenic under specific conditions(16)(17). Such research has shown that when specific gas like ammonia goes through the respiratory system via the nasal cavity, it causes variations to the normal flora of the nasal cavity (16).

Due to the observed wide use of ClO_2 badges, and due to the limitation of studies on the effect of this gas on pregnancy; this study primarily aimed to investigate the safety of inhaled low concentrations of ClO_2 (0.03 ppm as labeled by AirDoctor®) on pregnant rats and their fetuses through the possible changes in the physical parameter, biochemical parameters, and the possible changes on the microbiota of nasal cavity in pregnant rats. Moreover, the possibility of miscarriage in rats and the effect on the offspring of the rats were tested.

Materials and Methods

This study was approved by by Applied Science Private University Ethics Committee for the Care and Use of Experimental Animals in Education and Scientific Research – Faculty of Pharmacy (2021-PHA-43).

Experimental animals

Thirty-two female Wistar-Albino strain rats 10 to 12 weeks old were kept in standard clear-sided cages with a stable ecosystem (12/12 light-dark cycle, 25 C, and 50–60% moisture) and free access to food and water for two weeks.

Mating and gestation confirmation

The female rats were examined frequently by vaginal smear test to confirm they were in regular estrus periods (18). Then they were placed randomly with sixteen male Wistar-Albino rats, every two females with one male in the same cage for 48 hours to mate freely (19). Mating was confirmed by the presence of sperms in the vagina the next morning which was the baseline day (day-zero) of gestation, and 5 days later the pregnancy was tested by the persistence of diestrus (19). Overall, twenty-two female rats were confirmed pregnant while the other non-pregnant females were excluded from the study.

Experimental Design.

The pregnant rats were divided randomly into one test group and one control group, each of 11 rats Table 1. Every 3 to 4 pregnant rats from each group were kept in a separate cage and all cages for the same group were followed up in a separate room. The rats were kept in standard conditions related to light cycle, temperature, humidity, water, and food for the whole period of their gestation (around 21 days).

Treatment

Each cage of the test group rats had a badge of AirDoctor (Kiyou Jochugiku Co. Ltd, Japan). Which was hitched just above the cage by a few centimeters. AirDoctor® badge was activated (unsealed) at the time of hosting the pregnant rats in the cages just after confirming gestation (one-day gestation). The cages of rats of the control group were not exposed to AirDoctor in the other room.

Experimental samples and analyses

Blood samples

Blood samples were withdrawn from the retro-orbital sinus vein around 2 ml (20) of each overnight fasting rat in both groups (test and control) at two points of gestation during this study: day zero and day 18. The serum was separated by centrifugation (Biosan, USA), and stored immediately at −80°C in deep freeze (Qingdao Haier Biomedical Co., Ltd, China) until used according to ALT (Linear (Barcelona, Spain)), and creatinine (Spinreact, Barcelona, Spain) analysis methods (18)(23) leaflets.

Nasal cavity microbiota

Randomly, three female rats were selected. A nasal cavity swab was taken for each of them at day zero before exposure to AirDoctor and day 18 gestation after continuous exposure to AirDoctor. The DNA extraction and the 16s rRNA sequencing were followed according to (24,25) with slight modification. The nasal cavity swabs were freshly collected in antiseptic plastic Eppendorf centrifuge tubes then immediately frozen at −80°C and kept until the microbiota composition was analyzed. The process of DNA extraction was executed with G-Spin™ Total DNA Extraction Kit protocol (iNtRON Biotechnology). The DNA samples were shipped to the Molecular Research Lab (www.mrdnalab.com, MR DNA, Shallowater, TX) for rRNA sequencing by using the Illumina platform and then analyzed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In the final step, the Operational Taxonomic Units (OTUs) were taxonomically by BLASTn against a database obtained from RDPII and NCBI (https://www.ncbi.nlm.nih.gov, https://rdp.cme.msu.edu). Then the comparison in this study occurred after filtering out the relative abundance of <1%.

Weight and general observations

The pregnant rats were weighed at day zero and 18-day of gestation and the mortality among pregnant rats was recorded by balance HZT (Aadarsh, Mumbai).

The delivered pubs from both groups were counted and weighed; moreover, the health status of newborns was monitored by eye for two weeks.

Statistical analysis

All statistical analyses were conducted using IBM SPSS version 24 software. For the variables that had Shapiro-Wilk significant value, a non-parametric statistical analysis test was conducted, i.e. Mann-Whitney test and Wilcoxon Signed Rank test. Otherwise, independent samples T-test and Fisher-Exact test were followed.

Results

Pregnant rats

Average weight

The female rats of the two groups were weighed on day-zero and day 18 of gestation. The average weights at day zero were 197.73 gm and 199.09 gm for the control and AirDoctor groups, respectively. After 18 days of gestation, average weights reached 250.45 gm and 253.64 gm, respectively. No statistically significant difference was found between the weights of the two groups at each point. Figure 2.

Serum alanine aminotransferase (ALT) analysis

Mean serum ALT levels increased after 18 days of gestation, yet not significantly for both groups. For the control group, average serum ALT was 45.45 U/L at day zero elevated to 48.78 U/L at 18-day gustation (p= 0.643). In the AirDoctor group, the average serum ALT was 40.88 U/L at day-zero then elevated to 51.55 U/L at 18-day gustation (p= 0.341) Table 2.

Table 1. Study design

Groups before breeding	Status	Classification after breeding	Status	Treatments	Total number of pregnant rats
32 female Wistar-Albino rats	Healthy	Control group	Healthy pregnant	No intervention	11
		Tested group	Healthy pregnant	ClO_2 gas	11

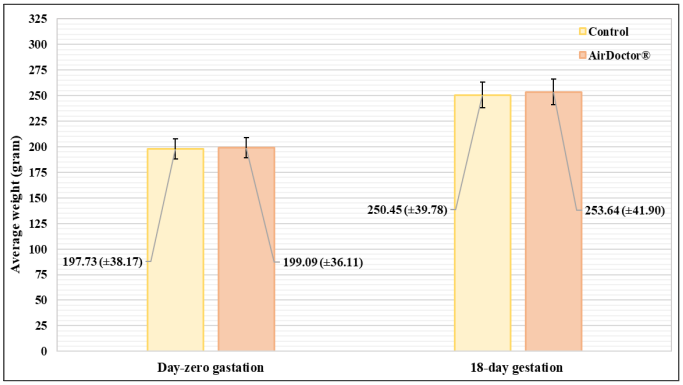


Figure 2. Average weight of the rats at day-zero and 18-day gestation for both groups.

Table 2. Comparative average ALT serum levels at day zero and day 18 of gestation for both groups Wilcoxon Signed Ranks Test (nonparametric)

The Groups	Day-zero U/L (±S.D.)	18-day gestation U/L (±S.D.)	*p*- Value
Control	45.45 U/L ((±18.14)	48.78 U/L ((±20.70)	0.643
AirDoctor®	40.88 U/L ((±14.89)	51.55 U/L ((±21.87)	0.341

Serum Creatinine analysis

The mean readings of serum creatinine for the control group at days zero and 18 were 1.42 mg/dl. For the AirDoctor group, the mean serum creatinine insignificantly (p = 0.952) decreased from 1.53 mg/dl to 1.16 mg/dl Figure 3.

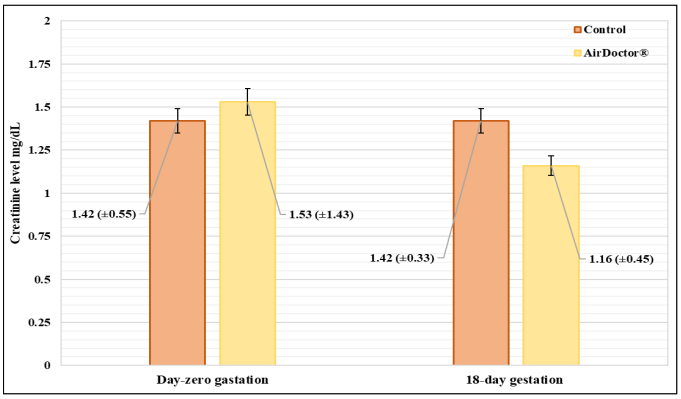


Figure 3. Comparative average Creatinine serum levels at day-zero and 18-day gestation for both groups.

Nasal cavity microbiota

Since the generated gas from the AirDoctor® passed through the respiratory system via the nasal cavity, probable changes in normal flora were examined.

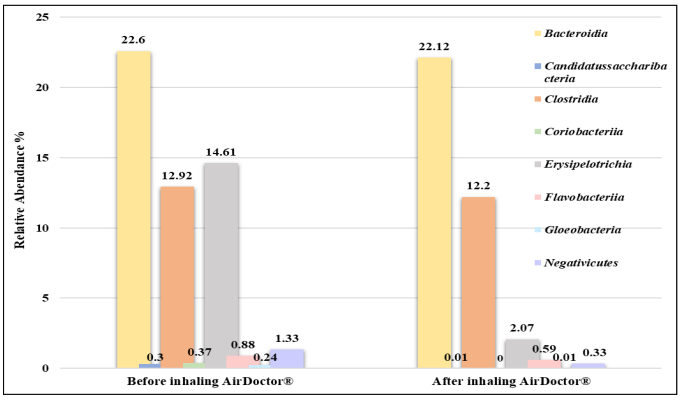


Figure 4. Relative abundance of bacterial classes which decreased after exposure to AirDoctor®.

Relative abundance of classes in nasal cavity samples

In the current study, the effect on the bacterial classes can be divided into two patterns. In the first one, a decrease in the relative abundance after exposure to AirDoctor® was noticed. For example, the relative abundance of *Bacteroides* was (22.6%) and after exposure to AirDoctor, it went down to (22.1%) which is the most abundant bacterial class. Similarly, the relative abundance of members belonging to *Erysipelotrichia* (14.6%), *Clostridia* (12.9%), and *Negativeicutes* (1.3%) decreased after exposure to AirDoctor to 2.1%, 12.2%, and 0.3%, respectively Figure 4.

The second pattern is composed of the bacterial classes whose relative abundance became higher after exposure to AirDoctor®. The most abundant bacterial class in this group was *Gammaproteobacteria* (28.9%) then became 39.7%. Also, *Bacilli* (7.8%), *Betaproteobacteria* (3.3%), *Alphaproteobacteria* (3.2%) and *Actinobacteria* (2.5%) increased after exposure to AirDoctor (8.3%, 3.4%, 5.72% and 3.8% respectively). On the other hand, bacterial classes like *Acidimicrobiia* (0.8%) were found to exist only after exposure to AirDoctor® Figure 5.

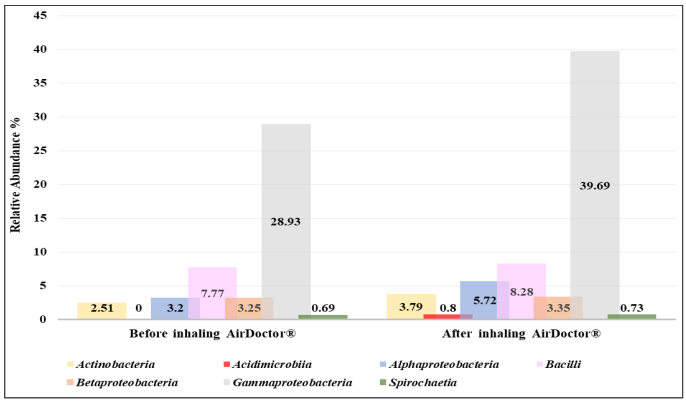


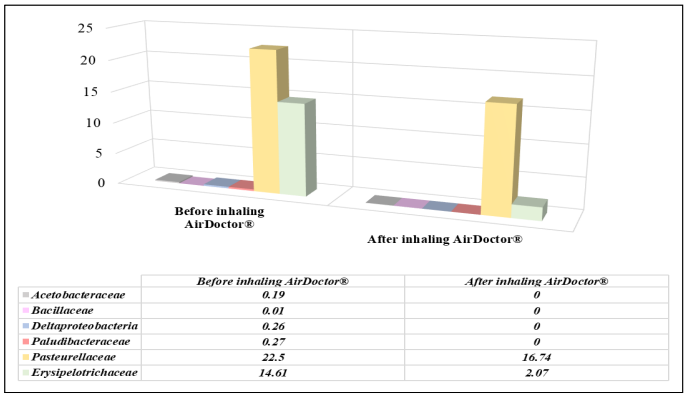
Figure 5. Relative abundance of bacterial classes which increased after exposure to AirDoctor®.

The number of bacterial classes that decreased in relative abundance after exposure to AirDoctor® is higher than the number of bacterial classes that increased after exposure to AirDoctor®.

Relative abundance of bacterial families in nasal cavity samples

The present results show that the bacterial family *Pasteurellaceae* had the highest relative abundance of other bacterial families, it started at 22.5% then after exposure to AirDoctor® became 16.7%. It was followed by *Erysipelotrichaceae* (14.6%), and its relative abundance decreased to 2.1% after exposure to AirDoctor®.

Moreover, bacterial families of *Bacillales*, *Acetobacteraceae*, *Deltaproteobacteria*, *Paludibacteraceae*, and *Eggerthellaceae* were found with negligible relative abundances, which disappear after exposure to AirDoctor® Figure 6.



	Before inhaling AirDoctor®	After inhaling AirDoctor®
Acetobacteraceae	0.19	0
Bacillaceae	0.01	0
Deltaproteobacteria	0.26	0
Paludibacteraceae	0.27	0
Pasteurellaceae	22.5	16.74
Erysipelotrichaceae	14.61	2.07

Figure 6. Relative abundance of bacterial families which decreased after exposure to AirDoctor®.

However, the increasing pattern after exposure to AirDoctor® contained *Prevotellaceae* (12.5%), *Moraxellaceae* (2.3%), *Pseudomonadaceae* (2.2%), and *Chromatiaceae* (0.3%), for which the relative abundance increased after exposure to AirDoctor® to 13.0%, 8.1%, 7.0%, and 6.0% respectively. Families of *Microbacteriaceae* and *Lamiaceae* with slight relative abundance appeared after the exposure to AirDoctor Figure 7.

Mortality among pregnant rats

The number of pregnant rats that survived and died was followed up during the length of the study. Mortality within the two groups before or at delivery was compared by using the Fisher-Exact test. Although there was one death in the AirDoctor® group and two deaths in the control group, it did not make the difference statistically significant (p= 1.00) Table 3.

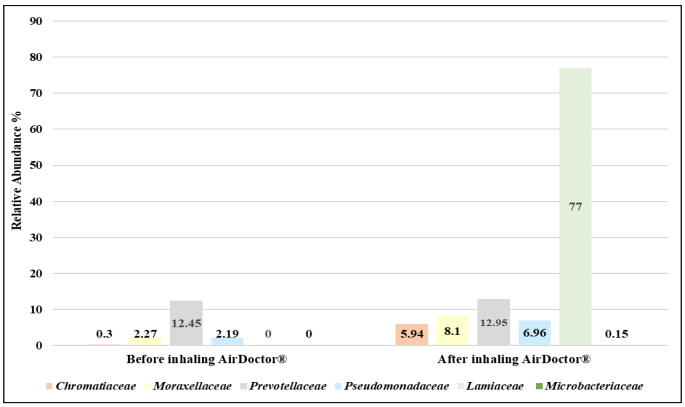


Figure 7 Relative abundance of bacterial families which increased after exposure to AirDoctor®.

Table 3. Comparative survival of the pregnant rats over the study period
(p = 1.00) " Fischer Exact Test "

The Groups	Total number	Alive till delivery	Dead before or at delivery
Control	11	9	2
AirDoctor®	11	10	1

Offspring

Average number of pups

The delivered pubs from both groups were counted and compared. No statistically significant difference was discovered regarding the average number of pubs per pregnant rat in the control group (n= 9) and the AirDoctor® group (n=10), (p= 0.362) Figure 8.

Average weight of a pup

The average weight of pups in the control group was higher (5.64 gm) than the pups of the AirDoctor group (5.33 gm). That difference was not statistically significant (p= 0.570) Figure 8.

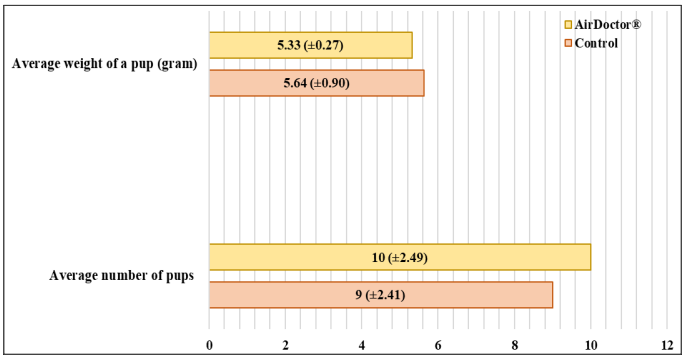


Figure 8. Pups' numbers and average weight for both groups.

General observations

By macroscopic examination, no teratogenicity was observed regarding the limbs, ears, eyes, mouth, or tail of the delivered pubs from the two groups, from the moment of birth to the age of two weeks.

Discussion

Prevention of viral and bacterial diseases is a hot topic that concerns the whole world against airborne diseases. ClO_2 acts as a biocide that kills most pathogens that cause disease. During the COVID-19 pandemic, the use of ClO_2 increased rapidly and in different forms of use due to a lack of specific treatment or prevention. Yet to date, no scientific evidence has been reported on using ClO_2 in the prevention of COVID-19 (3).

According to previous studies, using ClO_2 as an anti-infective is approved to be effective below the eight-hour average weighted mean exposure level (0.1 ppm) defined by the Occupational Safety and Health Administration (OSHA, U.S.A.) with significant margins of safety providing minimal risks (10)(6). Because exposure to high doses of ClO_2 has been shown high toxicity in animal models it is not safe for human consumption (3).

There may be negative and skeptical opinions by healthcare providers because there is a lack of awareness about the safety and efficacy of low-concentration ClO_2 in some countries where ClO_2 producing kits are used (7). In fact, ClO_2 inhalation can cause death if exposures are above the occupational exposure limit value (7).

Referring to a previous study, although 1,372 documents are talking about ClO_2 on PubMed (National Library of Medicine), there is a lack of evidence of the safety of ClO_2 during pregnancy. Some studies refer to the need to ensure that the concentration of chlorine dioxide can be safe at constant levels so that we can ensure the effects are beneficial and harmless (11).

In this study, none of the tested measures showed a negative effect of ClO_2 on pregnant rats during the gestation period. Miscarriage incidents did not happen, and no teratogenic remark was recorded upon macroscopic examination of the offspring for two weeks post-delivery.

According to Figure 2; the weight of rats was in the normal increase of pregnancy for both groups and was similar between them which was gradually increasing during the time of gestation for both groups with no statistically significant difference.

After the widespread use of AirDoctor® during the COVID-19 pandemic; it is necessary to investigate the safety profile, by considering potential hepatoprotective and nephroprotective effects of the studied serum. Thus, the serum levels of ALT were measured as markers of hepatotoxicity, and the serum creatinine levels were measured as an indicator of nephrotoxicity. These two biochemical parameters were assessed by measuring them for both groups on day zero and day 18 of gestation. The comparison was conducted intra and inter-between the two groups to investigate the safety profile of AirDoctor®. Referring to Table 2. serum hepatic marker ALT was found elevated in both groups of rats towards the end of gestation without a significant difference between the two groups, such elevation in serum ALT is expected due to uterine muscle contraction during labor (26). Thus, exposure to AirDoctor® did not increase serum ALT significantly, suggesting the absence of the negative effect of exposure to ClO_2 in low concentrations during pregnancy on the health of liver. Regarding serum creatinine level; in general, the serum creatinine level during pregnancy is decreased due to an increase in glomerular filtration rate (GFR) (27). According to Figure 3. In this study, during 18 days of gestation, the creatinine level for the control group was not increased or decreased; however, the creatinine level for the AirDoctor® was decreased. Although this result is statistically insignificant; it is worth noting that the exposure to AirDoctor® played a noticeable role in decreasing the level of serum creatinine after 18 days of gestation which may play a positive role in the case of nephroprotective.

According to 16s ribosomal rRNA sequencing, and after studying the bacterial classification (classes and families) and how they were affected by AirDoctor® on mating day (before the exposure) and after gestation (after the exposure), no significant differences were noticed regarding bacterial classes and families by using Wilcoxon-Signed test (all p-values were > 0.05).

In Figure 4-7**,** Referring to the bacterial some of them decreased after exposure to the AirDoctor® as *Bacteroidia* which is the highest abundance (gram-negative bacteria belonging to the phylum *Bacteroidetes*) (24), *Negativicutes,* and *Flavobacteria* (gram-negative bacteria)(28)(29). Other bacteria increased after exposure to AirDoctor® such as *Bacilli* (gram-positive bacteria of the *Firmicutes* phylum) (30) and *Actinobacteria* (gram-positive bacteria) (31) also increased after exposure to AirDoctor®. On the family levels, the highest relative decrease in abundance of bacterial families after using AirDoctor® was within *Pasteurellaceae* a large family of gram-negative bacteria found mostly in the upper respiratory tract (32). *Paludibacteraceae* is a family of gram-negative that belongs to the *Bacteroidete*s phylum (33) and is one of several bacterial families that disappeared after exposure to ClO_2. At the same time, many bacterial families appeared after exposure to ClO_2, for instance, *Microbacteriaceae* and *Acidimicrobiia* gram-positive bacteria belong to *Actinobacteria*) (34) (35).

Chlorine dioxide gas has free radicals and unpaired electrons. Accordingly, it has a broad-spectrum antimicrobial activity (36). The mechanism of antimicrobial activity of ClO_2 is due to its ability to desaturate proteins by inducing covalent oxidative modification. It reacts with proteins and amino acids in the bacterial cell structure and alters their chemical characteristics which then destroy it (36). The inactivation of microbes by (ClO_2) is caused by oxidative modification of their tryptophan and tyrosine residues (7). (ClO_2) can react faster with cysteine and methionine than with tyrosine and tryptophan, because the first two amino acids contain sulfur atoms (two aromatic amino acids) which assumes the antimicrobial effect of chlorine dioxide(37). To explain why in this study almost all the gram-negative bacteria disappeared or decreased after exposure to AirDoctor®, chlorine dioxide solution is more effective against gram-negative than gram-positive (38).

It is interesting to mention that mortality and morbidity throughout the world are due to gram-negative bacteria; which can cause respiratory tract infections(7). The outer cell membrane is a distinguishing feature of gram-negative bacteria and chlorine dioxide was found to increase the permeability of the outer membrane and the cytoplasmic cell membrane thus resulting in release the of vital nuclear material and then loss of cell activity or death (39). Therefore, that is why chlorine dioxide is effective against gram-negative, so using low concentrations of chlorine dioxide against gram-negative bacteria is a feasible method without causing adverse effects (7).

According to our result of 16s ribosomal RNA sequencing, the number of bacterial classes that decreased after exposure to AirDoctor was 8 compared to 7 classes that increased after exposure to AirDoctor®. Moreover, the number of bacterial families that decreased after exposure to AirDoctor® was 6 compared to 6 families that increased after the exposure. There was no noticeable difference before and after the use of AirDoctor®, so this explains why the results of the statistical analysis were not significantly different.

Finally, in Figure 8, as for delivery and offspring, the two groups followed almost the same path in terms of pups' weight and number without significant differences where the number of pups and their weight were within the normal range and their health for two weeks old was good. Thus, these indicate that there were no negative effects of ClO_2 on fetuses.

Conclusion

Finally, As seen in many researches, chlorine dioxide is one of the best germicides and disinfectant products available in the market; also, it has a fast-acting oxidizer with a lower concentration than any other chlorine disinfection. There are several forms in which chlorine dioxide is sold in this study we use the prepackaged granules that were used in the badge; under the commercial name AirDoctor. Based on the research aim, AirDoctor® demonstrated a statistically insignificant effect in pregnant rats in all parameters (physical and biochemical). Also, there is no effect on the duration of pregnancy and no miscarriages were reported. Moreover, after monitoring the fetus there are no abnormalities shown after two weeks of delivery. According to the possible changes in the microbiota of the nasal cavity, the results show that AirDoctor® does not affect negatively the normal flora in the respiratory system; however, it is more effective in decreasing or disappearing on gram-negative than gram-positive. Ultimately, the safety of AirDoctor® cannot be doubted, but up to date, there are no clinical studies on pregnancy confirming safety, the duration of use, and the route of administration.

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Author contribution

Wild Ali, Mai, Data collection and processing, Literature search, Writing manuscript. Darwish Elhajji, Feras, Conception, Design, Supervision, Resources, Critical Review. Al-Najjar, Mohammad, Conception, Design, Resources, Materials, Critical Review. Alshaer, Shatha, Data collection and processing, Analysis and interpretation, Writing manuscript. Majzoub, Mohammad Design, Analysis and interpretation.

Conflict of Interest and Funding

This research was partially funded by the Applied Science Private University grant number DRGS-2012-2013-5. It was part of a master's degree thesis by the first author and the supervisors and colleagues. The sponsors had no role in the design, execution, interpretation, or writing of the study. The authors have no conflict of interest with anybody.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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