Post-coronavirus disease 2019 frequent use of disinfectants and sanitizers affect lung tissue: experimental study

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Background/aim
Regular and excessive use of general household disinfectants and hand sanitizers has increased since coronavirus disease 2019 (COVID-19) hit as per World Health Organization (WHO) recommendations. However, existing knowledge about hypochlorite use as a disinfectant is inadequate for the applied experimental conditions are generally not translated to real life. This study explores the effect of the regular use of the commonly recommended disinfectants such as alcohol-based solutions and 0.1% hypochlorite on the lung and liver tissues of rats.

Materials and methods
Sixteen Wistar rats were assigned to two housing conditions, first group cages were cleaned regularly with tap water. The second group was exposed to regular sanitization of the cages twice daily for 2 months using 70% alcohol followed by 1% hypochlorite solution, 30 min apart. Serum redox state was evaluated and serum liver enzymes were assessed. Lung and liver tissues were examined biochemically for inflammatory markers such as IL-1B, NF-kB, VEGF, and oxidant biomarkers such as MDA and antioxidant markers including GSH, SOD, and GPx. A histological examination was performed.

Results
Serum liver enzymes, antioxidants in serum or liver tissues and the inflammatory biomarkers in liver tissue were insignificantly changed, while lung tissue was inflamed and proceeded to fibrotic changes and the inflammatory biomarkers of IL-1β and NF-kβ, and VEGF of lung tissue were significantly elevated ($P < 0.05$) after surface disinfectant exposure to alcohol followed by 0.1% hypochlorite solution for two months. Moreover, the GSH and GPx levels were significantly reduced ($P < 0.05$), while the MDA level was significantly increased ($P < 0.05$) in the serum and lungs of a disinfectant exposed group of rats.

Conclusion
Frequent and excessive exposure to alcohol followed by 0.1% hypochlorite solution even as recommended can pose a risk to the respiratory system. Their application in cleaning routine should be wisely considered.

Keywords:
household disinfectant, hypochlorite, inhalation toxicology, respiratory diseases

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic has sparked a worldwide panic due to infection that can happen through airborne transmission through inhaling viral particles suspended in the air, depositing infectious droplets from exhaled breath directly on mucous membranes, or indirect contact with contaminated secondary surfaces, such as hands or fomites [1]. Since the outbreak of the most recent Covid-19 pandemic, there has been a growth in the regular and excessive usage of general household disinfectants and hand sanitizers. In hospitals, residences, and several public locations, disinfectants are frequently used. Additionally, they are included in skin cleansers.

The World Health Organization (WHO) recommended alcohol-based sanitizers to disinfect hands and 0.1% hypochlorite for surfaces since studies have shown that these formulations effectively inactivate viruses [2,3]. An incidence of someone taking a disinfectant during a psychotic outburst out of fear of contracting the Covid-19 infection was documented since so many people followed this advice with terror and misused disinfectants in their daily lives [4]. Out of anxiety, this behavior persisted after the COVID crisis, and roughly 70% of the general population changed their washing and sanitizing routines [5]. Inhalant-induced asthma was linked to regular, low-level exposure to cleansers [6]. Furthermore, women population exposed

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to non-professional household cleaning agents had asthmatic and bronchial inflammatory symptoms [7].

Studies on occupational exposure that concentrated on asthma aggravation provide the majority of the evidence for the harmful effects of cleaning. Frequent daily low-level exposure to irritants including chlorine, ammonia, hydrochloric acid may be involved in irritant-induced asthma [8]. However existing knowledge is inadequate. Most experimental data were taken from investigations that were conducted in inhalation chambers for a brief period of time and largely involved quaternary ammonium compounds [7], thus makes drawing a clear connection between the study's results and actual life challenging.

The present study aims to explore the effect of the regular use of the common recommended disinfectants such as alcohol-based solutions and 0.1% hypochlorite on the lung and liver tissues of rats after daily exposed to regular sanitization for 2 months.

Material and methods

Chemicals and reagents

Commercial Sodium Hypochlorite solution was used at 0.1% concentration (Clorox, Egypt) and commercial ethyl alcohol at 70% dilution (El-Gomhoreya, Egypt) were purchased and used in the current study. Liver enzymes colorimetric kit (CAT#1034), Catalase (CAT) (CAT#2517), Superoxide disumutase (SOD) (CAT#2521), and glutathione peroxidase (GPx) (CAT#2524) were procured from Biodiagnostic, Egypt. Enzyme-Linked Immunosorbent Assay (ELISA) kit for interleukin-1β (IL-1β) (CAT#E-EL-R0012), interleukin-1 β (IL-1 β) (CAT#), vascular endothelial growth factor (VEGF) (CAT# E-EL-R2603) and nuclear factor kappa-β (NF-k β) (CAT# E-EL-R0674) were obtained from Elabscience biotechnology, China.

Animals

Sixteen Adult male Wistar rats (220-300 g, NRC, Egypt) were housed in the animal vivarium of the National Research Centre, Dokki, Egypt. Animals were acclimatized for six days in standard cages (4/cage) with diet pellets and water adequately available. The vivarium keeps an automated controlled environment: temperature, 23±3°C; relative humidity, 45%–70%; 12 h light/12 h dark cycle; lights on 7:00 a.m. All animal protocols described in this study followed the NIH publication (No. 8023, revised 1978) and were approved by the Institutional Animal Care and Use Committee of the National Research Centre, Egypt.

Experimental design

Two groups of rats were assigned to the study protocol (each of 8 rats). First group was housed normally and placed in clean cages regularly for the whole experimental period. The second group was exposed to regular sanitization of the cages while they were inside twice daily for two months using 70% alcohol followed by 0.1% hypochlorite solution, 30-min apart. At the end, blood samples were taken under light anesthesia, lung and liver tissue were extracted. Part of each tissue was weighed and homogenized in ice-cold 0.9% physiological saline, centrifuged and the supernatant was stored in −80°C for biochemical examination. The remaining lung tissue was stored in 10% para-formaldehyde solution for histological examination.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experimental procedures performed in the study were in accordance with the principles expressed in the Declaration of Helsinki. This study has been approved by the local Ethics Committee of National Research Centre, Cairo, Egypt with approval number 3231/11/2/2022.

Blood and tissues sampling

At the end of the exposure period, blood samples were collected from the retro-orbital plexus under light anesthesia. Thereafter, rats were euthanized by cervical dislocation. Liver and lung tissue were dissected and cut into parts. one part was put in 10% formalin solution while other parts were homogenized in ice-cold phosphate buffer saline (pH 7.4) for further biochemical assessments and stored at −80°C till processing.

Biochemical methods

Serum levels of Aspartate transaminase (GOT), Alanine transaminase (GPT), Serum and tissues levels of Malondialdehyde (MDA) and Glutathione-S-Transferase (GSH), were determined by the colorimetric method, using kit of Biodiagnostic, Co., (Cairo, Egypt), according to the manufacturer's instruction. The lung and liver superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were evaluated by the use of colorimetric kits of Biodiagnostic Co, (Cairo-Egypt) following the manufacturer's instruction, while lung and liver pro-inflammatory marker IL-1 β, VEGF and NF-kβ were

evaluated using ELISA kit, according to the manufacturer's instruction of Elabscience Co, (China).

Histological examination

After 48 h from fixation in 10% formalin, the specimens of lung and liver were washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Paraffin blocks were sectioned into five-micron thick sections, mounted on clean slides and stained with hematoxylin-eosin (H and E) for microscopic evaluation [9].

Statistical analysis

All data are presented as mean±standard deviations. Significant difference was determined by Student's unpaired *t*-test at *P* less than 0.05 and *P* less than 0.01 levels of significance using GraphPad Prism version 9.3.1 for Windows 64-bit, GraphPad Software, San Diego, California USA.

Results

Results of serum liver enzymes, MDA and GSH

Compared with the nonexposed control group, those group of rats exposed to the disinfectant had normal activity in GOT and GPT liver enzymes, on the other hand, rats exposed to disinfectants had considerably higher serum MDA levels (38.3±2.4 nmol/ml) than control rats (16.3±0.5 nmol/ml), at *P* value less than 0.05, while the GSH content of the rats' serum was significantly lower than that of the controls (0.91±0.01 vs. 0.99±0.01 μmol/ml, $P < 0.05$) (Table 1).

Table 1 Serum Liver enzymes GPT and GOT after exposure of rats to surface disinfectants

	Un-exposed group	Exposed group
GPT (IU/L)	37.5±1.2	39.1±2.1
GOT (IU/L)	33.4±2.2	32.0±1.6
MDA (nmol/ml)	16.3±0.5	38.3±2.4*
GSH (μmol/ml)	0.91±0.01	0.99±0.01*

All data are shown as mean±S.E. *Significance difference than un-exposed group at *P* value less than 0.05, using student's unpaired *t*-test.

Results of oxidative stress and inflammation markers in liver and lung tissues

The present results tabulated in (Table 2) revealed that the lipid peroxides indicator MDA was measured to be 18.2±0.92 and 18.18±0.84 nmol/mg protein in the liver of control and disinfectant exposed groups respectively, which are insignificantly differences at *P* value greater than 0.05. Likewise, the levels of antioxidant molecule GSH, and the antioxidant enzymes, SOD and GPx in liver tissues were not affected by disinfectant exposure, at *P* value greater than 0.05 comparing to the control group (Table 2).

On the other hand, the lung tissue showed induced level of lipid peroxidation in disinfectant-exposed group compared with control group (10.7±0.7 vs. 6.2 ±0.5 nmol MDA/mg protein, $P < 0.05$). The GSH content of lung tissue was depleted after disinfectant exposure (0.97±0.01 vs. 1.4±0.1 μmol/mg protein, $P < 0.05$). In addition, GPx activity was considerably decreased than Control lung tissue (0.45± 0.1 vs. 0.33±0.02 U/mg protein, $P < 0.05$), while SOD activity was not altered after exposure to regular sanitizers (2.6±0.3 vs. 2.4±0.8 U/mg protein $P > 0.05$), as displayed in (Table 2).

ELISA analysis of lung tissue revealed significant increment of inflammatory mediators of IL-1β (58.1 ±3.3 vs. 12.7±0.4 pg/mg protein, *P* value < 0.05) and in the levels of NF-kβ (4.5±0.1 vs. 3.3±0.1 pg/mg protein, *P* value < 0.05) after disinfectant exposure compared with the control group. Likewise, the remodeling factor VEGF was induced in lung tissue of disinfectant exposed group (6.34±0.13 pg/mg protein, $P < 0.05$) compared with the nonexposed group (2.34±0.07 pg/mg protein). On the other hand, liver tissue was not affected and the measured

Table 2 Lung and liver redox capacity after exposure of rats to surface disinfectants

	Un-exposed group		Exposed group	
	Liver	Lung	Liver	Lung
MDA (nmol/mg protein)	18.21±0.92	6.20±0.45	18.18±0.84	10.71±0.66*
GSH (μmol/ mg protein)	0.89±0.02	1.38±0.1	0.90±0.02	0.97±0.01*
SOD (U/mg protein)	4.65±0.27	2.57±0.13	5.16±0.15	2.35±0.38
GPx (U/ mg protein)	0.53±0.01	0.45± 0.01	0.60±0.07	0.33±0.01*
IL-1 β (pg/mg protein)	32.60±0.98	12.72±0.36	35.16±1.34	58.14±3.30*
NF-kβ (pg/mg protein)	2.52±0.09	3.34±0.09	2.70±0.07	4.46±0.11*
VEGF (pg/mg protein)	3.20±0.07	2.34±0.07	3.56±0.14	6.34±0.13*

All data are shown as mean±S.E. *Significance difference than lung un-exposed group at *P* value less than 0.05, using student's unpaired *t*-test.

IL-1β and NF-kβ were of comparable levels compared with un-exposed group (Table 2).

Histological evaluation of lung and liver tissue

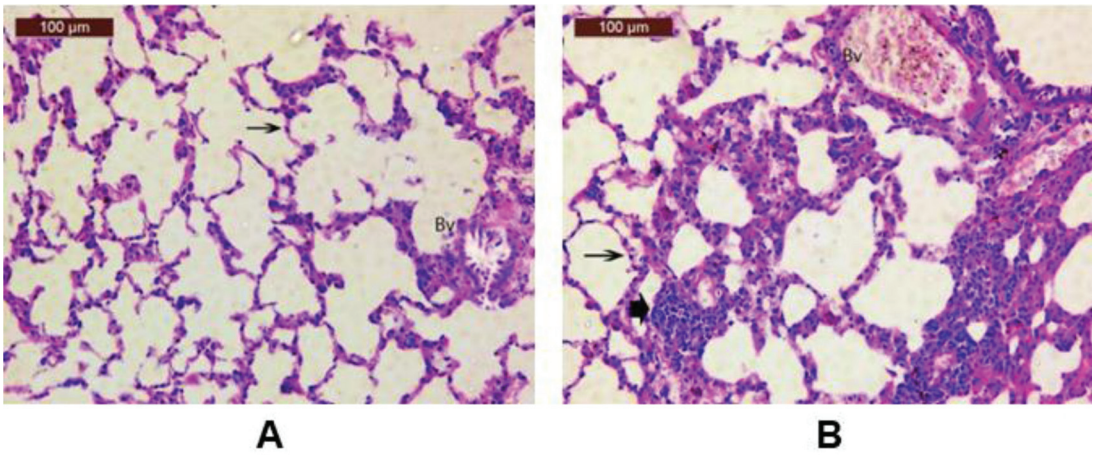
Microscopic examination of lung sections of control rats showed the lung alveoli (air sacs) with normal histological structure. The walls of the lung alveoli appeared thin and lined with thin wall of flat epithelial cells (Fig. 1a). However, the disinfectant exposure induced marked thickening of the alveolar wall, dilated and congested blood vessel with thick wall. Multiple large areas of hemorrhage and mononuclear cellular infiltration could be seen (Fig. 1b).

The liver sections in the control group showed a normal histological picture of central vein at the center of the lobule surrounded by the hepatocytes between the strands of hepatocytes. The hepatic sinusoids are displayed and distinct nuclei are observed as shown in (Fig. 2a). On the other hand, liver tissue after disinfectant inhalation revealed a nearly normal histological structure, normal central vein except dilated hepatic sinusoids and activated Kupffer cells (Fig. 2b).

Discussion

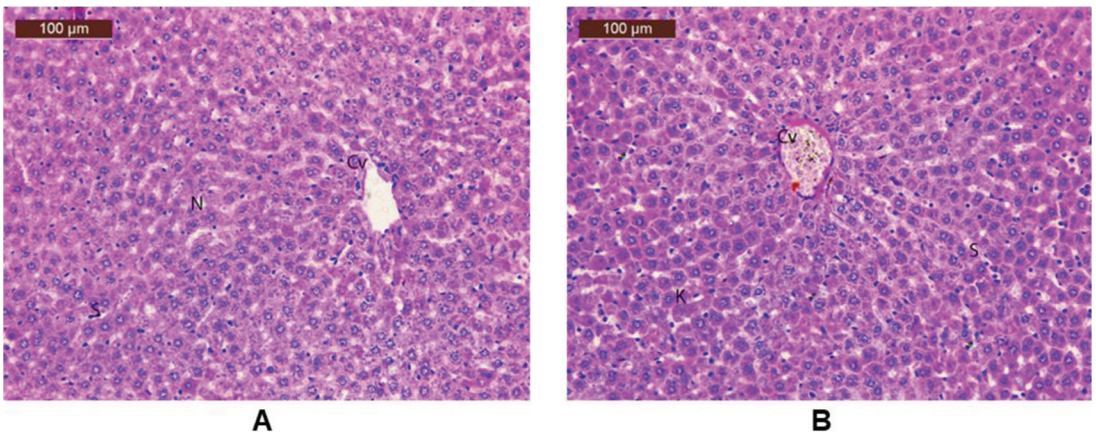
The current investigation showed inflammatory and oxidative changes in lung tissue and blood after exposure of rats to surface disinfectants via common practice specially after the COVID-19 pandemic. Our study reported increased inflammatory markers (IL-1

Figure 1



A) lung tissue of un-exposed control rats, showing the lung alveoli (air sacs) with normal histological. The walls of the lung alveoli appeared thin (arrow) structure and blood vessels (Bv). B) lung tissue of disinfectant-exposed rats, showing marked thickening of the alveolar wall (arrow), dilated and congested blood vessel (Bv). Multiple large areas of hemorrhage and mononuclear cellular infiltration could be seen (arrowhead) (H and E x 200).

Figure 2



A) Liver tissue of un-exposed control group showing normal hepatic architecture, central vein (CV), blood sinusoids (S) and nucleus (S) of normal appearance. B) liver tissue of disinfectant-exposed rats showing almost nearly normal histological structure, normal central vein (Cv), except dilated hepatic sinusoids (S) and activation Kupffer cells(K) (H and Ex200).

β, NF-kβ), VEGF and lipid peroxidation marker, MDA in lung tissue. On the other hand, lung antioxidant molecules such as GSH and GPx were reduced. Normal liver functions were noticed.

Previous animal exposure studies reported asthma cases or lung injury related to surface cleaned with common disinfecting agents particularly quaternary ammonium compounds including benzalkonium chloride and didecyldimethyl a-mmonium chloride [7]. Pulmonary damage and inflammation were reported after exposing the rats to these compounds by chamber inhalation or intratracheal instillation [10,11].

The current study exposed the animals through wiping of the cage by the tested disinfectants without exposing to aerosolized preparations or containment in a fumed chamber to mimic the actual disinfection common practice. Furthermore, the used disinfectants; sodium hypochlorite and alcohol, were the most common and the recommended for surface disinfection for protection against SARS-COVID-19 transmission.

Assessment of the redox capacity of liver and lung tissues revealed that liver was not affected by the exposure to surface disinfectants but lungs had a shift towards oxidized state. In the current study, GSH and GPx were inhibited after exposure to disinfectants. GSH is a key factor in antioxidant activity of a tissue, its reservoir is essential to the innate defense mechanisms enabling reduction of any oxidant to protect the tissue [12]. SOD enzyme is the first line of tissue defense against oxidative stress, whereas GPx detoxifies xenobiotic-generated peroxides by the help of the reducing capacity of GSH [13].

The observed inhibition of GPx activity indicates an excessive production of free radicals and peroxides [14], an observation supported by the depletion of the antioxidant GSH molecule and the induction of MDA, the lipid peroxide marker. MDA is reliable for assessment of membrane lipid peroxidation and the involvement of free radical damage to tissues. Hypochlorite is a powerful oxidant and responsible for the oxidation of the small thiol molecules such as GSH and formation of downstream radical products [15,16]. Membrane lipids are usually protected by GSH, excessive accumulation of hypochlorite oxidizes the GSH molecules and alter the redox state of the tissue. Lung injury in response to hypochlorite exposure mediated by peroxide products accumulation and GSH depletion was recorded in isolated rabbit lung [17].

Pulmonary inflammation occurs in a cascade of events after the redox state imbalance as a defense mechanism of tissue to prolonged oxidative stressful conditions [18]. Oxidative stress in pulmonary diseases is associated with activating inflammatory gene expression. NF-κβ regulates cytokine activity in the airway during inflammation [19]. The Nrf2/HO-1 signaling pathway is involved in the inflammation-induced pulmonary reaction [20]. The increased interleukins and NF-κβ in lung tissue is usually associated with fibrotic changes [21]. In the current study, exposure to regular home cage disinfection caused upsurged pulmonary inflammatory markers, IL-1B and NF-κβ in accordance with the fibrotic changes observed in histological examination. Hypochlorite was associated with workplace asthma in professional cleaners and its mechanism relies on oxidation of lung proteins. In rats exposed to hypochlorite for 15 days by nebulization, hypochlorite caused increment of lung IL-1B along with inflammatory cell infiltration [22].

The present investigation showed increased expression of VEGF in lung tissue. Pulmonary pro-inflammatory markers trigger overexpression of the vascular remodeling factor; VEGF necessary for lung architecture to drive abnormal angiogenesis in lung and may develop lung cancer [23]. Angiogenesis is an important pathogenesis behind asthma and other lung disorders [24]. VEGF enhanced activity is related to induced pulmonary fibrosis and subpleural fibrotic changes through pleural barrier damage and increased permeability [25]. A previous report showed increased VEGF immunostaining in lung tissue after exposure of adult rats to nebulized 4% hypochlorite solution for 4 days [26]. Mice exposed to aerosolized chlorine for 5 min daily expressed elevated VEGF and IL-6 in the bronchoalveolar fluid [27]. The histological findings confirmed the biochemical changes where vascular congestion and inflammatory reactions in lung tissue were evident in the rats exposed to hypochlorite excessive use in cleaning.

Liver enzymes were not changed in the disinfectant-exposed rats as well as the oxidant processes in liver tissue. The histological study showed no signs of hepatic inflammation. Liver enzymes are well correlated with the health of liver tissue. Serum GPT and GOT were reported to be indicators of

hepatocellular inflammations and tissue GSH, MDA, GPx, SOD are good representatives for oxidative status of the tissue [28]. No reports were found related to the effect of inhaling alcohol or sodium hypochlorite on liver tissue. The presented results showed no alteration in liver functions or oxidative changes in hepatic tissue after exposure to both disinfectants.

Conclusion

Long term excessive surface disinfection may trigger pulmonary inflammation and deposit fibrotic changes in lung tissue through upregulating cytokines and related angiogenesis morphological changes, thus careful and wise use should be highly recommended particularly in respiratory infection threat conditions.

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Nil.

Conflicts of interest

The authors declare no competing interest.

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