Safety of 0.5% hydrogen peroxide mist used in the disinfection gateway for COVID-19

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Abstract

Hydrogen peroxide (H_2O_2) is a reactive chemical used in a wide range of applications. Most importantly, it is used for sterilization process in health care environment. In the present study, safety assessment of 0.5% of H_2O_2or its mist intended to be used in the disinfection gateway for Covid-19 was evaluated. Skin irritation and repeated dose inhalation toxicity studies were carried out in rabbits and rats respectively. Skin irritation study revealed no observable oedema and erythema in rabbits after tropical application of 0.5% H_2O_2. Wistar rats (both male and female) were exposed (whole body exposure) to0.5% of H_2O_2 mist, at a concentration of 11.022 (low dose-2 min exposure), 22.044 (medium dose-4 min exposure) and 55.11mg/kg (high dose/high doe recovery-10 min exposure) body weight, daily for seven days. Rats in the high dose recovery group (55.11mg/kg-10 min exposure) were kept for another 7 days without any exposure. A toxicological evaluation was done based on general health parameters, haematology, serum biochemistry, gross necropsy and histopathological data. The results of the study indicated that there was no skin irritation potential induced on exposure of 0.5% of $H_2O_2$to rabbits. Similarly, the inhalation toxicity of 0.5% of H_2O_2 mist, imparts no evidence of haematological, biochemical, gross pathology or histopathological abnormalities in rats. Further, at the laboratory condition stimulated, the NOEL was found to be 55.11mg/kg body weight. Hence, the present study concluded that 0.5% H_2O_2 or its mist used in the disinfection gateway for COVID-19, failed to induce any skin irritation in rabbits or inhalation toxicity in rats.

1. Introduction

Hydrogen peroxide, colourless liquid easily decompose to oxygen and water in the presence of most metals and alkaline solutions. Itwas first synthesized by Louis Jacques Thénard in 1818 by reaction of nitric acid with barium peroxide. Hydrogen peroxide (H_2O_2) generally used and available in concentrations ranging from 3%-90%.H_2O_2, an oxidizing agent is actively used in wide range of applications, mainly in medicinal and house hold purposes (3–30%). Industries mostly use concentrated hydrogen peroxide (30–70%) for the purpose of bleaching (Ernstgard et al. 2012). Aqueous solutions of hydrogen peroxide in the range of 3–6% in water demonstrate broad-spectrum antimicrobial activity depending on the concentration and exposure time; hence it is also included in cosmetics, toothpaste, and detergents (McDonnell, 2009). H_2O_2both in liquid and gaseous form is widely used for biocidal applications. In health care facilities, vaporized hydrogen peroxide is used in sterilization of medical devices (Rutala and Weber 2015) and hydrogen peroxide-based automated room disinfection systems are utilized in pharmaceutical environments (Murdoch et al. 2016). Recently vaporized hydrogen peroxide (VHP) as a rapid sterilization technology is used as broad spectrum antimicrobial with virucidal, bactericidal, fungicidal and sporicidal activity (Lerouge et al. 2012). Virucidal effect of hydrogen peroxide vapour disinfection against poliovirus, rotavirus, adenovirus, MNV1 and influenza A virus has been documented (Tuladhar et al. 2012). Concern raised on the toxic effect of hydrogen peroxide due to its wide spread application. It has been documented that hydrogen peroxide once entered the blood circulation; it is rapidly decomposed in blood and remains unavailable systemically. Hydrogen peroxide induces toxicity depending on the route and concentration of

exposure. The permissible exposure limit as per Occupational Safety and Health Administration (OSHA) is found to be 1 ppm (averaged over an 8-hour work shift) (ATSDR, 2012). The 8 hour limit value of hydrogen peroxide was decreased from 1ppm to down to 0.05ppm by MAK commission. Among the routes of exposure (Ingestion, inhalation and dermal contact), inhalation is considered to be the major route for gaseous form exposure resulting in toxicity.

Studies on human have reported, industrial workers exposed to hydrogen peroxide 1.2 to 2.4 ml/m3 with peak exposures of 8 ml/m^3 caused irritation of the eyes and throat, nasal congestion, coughing and asthma symptoms revealing severe signs of inhalation toxicity (Hartwig et al. 2019). A Animal studies have reported, dermal LD_{50} of 90 % hydrogen peroxide solution, between 700 and 5000 mg/kg body weight in various species, among them the rat was the least sensitive (EC, 2003).Earlier studies also reported thatworkers exposed to H2O2 concentrations, less than 1 ppm showedrough skin on the hands and decolorized hairs (Suenaka et al. 1984). Studies on rodents documented that the oral LD_{50} of hydrogen peroxide (30%-70%) is found to be 694–1270 mg/kg body weight in rats (EU, 2012). In a 28-day, study Wistar rats exposed to hydrogen peroxide vapour in concentrations of 0, 2, 10 or 25 ml/m^3 for 6 hours per day and 5 days per week (nose-only exposure) showed concentration depended lung toxicity (ECIC, 2002).Studies also documented hydrogen peroxide as a well-known irritant to the mucous membranes and the airways (Watt et al. 2004). Even though numerous studies on hydrogen peroxide are available in literature, consistent and accurate toxicity studies remain elusive. Recently, exploiting virucidal, bactericidal and disinfectant activity of 0.5% of hydrogen peroxide mist, a disinfectant gate way was developed intended to be used for Covid-19 to control the extent of exposure to the viral load (Krishnan et al. 2020). In the present study, safety assessment of 0.5% H_2O_2 mist used in the disinfection gateway for COVID-19 was evaluated for skin irritation and repeated dose inhalation toxicity studies in New Zealand white rabbits and Wistar rats.

2. Materials And Methods

2.1 Animals

Wistar rats, both male and female of 8 weeks old with body weight range 170–250g was selected for inhalation toxicity study and New Zealand white rabbit weighing 2.0-2.6 kg was chosen for skin irritation test. The animals were selected from an inbred colonymaintained under the controlled conditions of 22 ± 3oC, humidity of 30–70 % and 12 h photoperiod with sterile food and water.

Experiments were performed after obtaining approval by the Institutional Animal Ethics Committee. The animal care and handling were according to the The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guide-lines for animal experimentation.

2.2 Skin irritation

Skin irritation potential was carried out as per International organization for standardization (ISO 10993-10, 2010). Three New Zealand white rabbits were used for the study. 0.5% H_20_2 solution (3%H_20_2IP-Pharmacy

grade) and physiological saline (control) were exposed topically on the upper left and right hand side of the clipped skin of 3 Rabbits (0.5mL/site). Application sites were covered with occlusive bandage for 4h.The test sample and control application sites were observed at 1h, 24h, 48h and 72h after removal of patches and observed for any tissue reactions. Skin irritation responses of rabbits were scored and recorded as ISO 10993-10.

2.3 Repeated-dose inhalation toxicity

Repeated-dose inhalation toxicity study was performed based on OECD guidelines 412 (OECD, 2018). Wistar rats of either sex (20 male and 20 female) were randomly assigned to five different groups: low dose, medium dose, high dose, high dose recovery and control group respectively (n = 4). Wistar rats (both male and female) were exposed (whole body exposure) to 0.5% of H_2O_2 mist (2–10 micron diameter), at a concentration of 11.022 (low dose-2 min exposure), 22.044 (medium dose-4 min exposure) and 55.11mg/kg body weight (high dose/high dose recovery-10 min exposure), daily for seven days. Rats in the high dose recovery group (55.11mg/kg-10 min exposure) were kept for another 7 days without any exposure. Control animals received only normal environmental conditions or exposure. All animals in each group (control, low, medium, and high) were sacrificed after 7 days. Recovery group animals were kept further 7 more days and sacrificed on 14th day.The justification for selection of dose/exposure is based on the multiple dose of human exposure (dose human exposure X6, X12 and X30 times) as shown in Table 1. All animals were subjected to daily observations for mortality and behavioural changes. Body weight of the animals before and after exposure was also recorded.

Table 1
Experimental design: Repeated dose inhalation toxicity of 0.5% H_2O_2 mist

No	Group	No of animals (Rats)		Duration of exposure	Dose mg/kg body weight of H_2O_2 mist
		Male	Female		
1	Control	4	4	0 min	0
2	Low dose	4	4	2 min	11.022mg/kg
3	Medium dose	4	4	4 min	22.044mg/kg
4	High dose	4	4	10 min	55.11mg/kg
5	High dose recovery	4	4	10 min	55.11mg/kg

2.4 Body weight

In the repeated-dose inhalation toxicity study, the body weight of Wistar rats exposed to hydrogen peroxide mist (0.5%) and control animals were recorded before and after completion of exposure.

2.5 Haematology

Wistar rats were anesthetized (diethyl ether) and blood was collected from retro-orbital venous plexus in sample tubes containing ethylene diamine tetra acetic acid (EDTA) and examined for the following parameters: white blood cell counts (WBC), red blood cell counts (RBC), haemoglobin concentration (Hb), haematocrit (HCT), MCV, mean corpuscular volume (MCHC) and platelets (PLT) using a haematology analyser (Abc VET, New Delhi).

2.6 Serum biochemistry

Blood samples were collected from retro-orbital venous plexus of rats and allowed to clot. The serum samples of both 0.5% of H_2O_2 mist exposed and non-exposed was separated after centrifugation at 3,000 rpm for 5 min. The serum was subjected to use for the estimation of alanine amino transferase (ALT), aspartate amino transferase (AST), Alkaline phosphatase (ALP), Triglycerides, albumin, total protein, total bilirubin, creatinine, glucose, cholesterol and urea using an automated biochemical analyser (ERBA XL 300,Germany) and as per the manufacturer's instructions.

2.7 Gross necropsy

At the end of observation period, all the animals in control and test groups were sacrificed by cervical dislocation. All the animals were subjected to gross pathological evaluations. Organs, such as lungs, cerebrum, cerebellum, liver, spleen, pancreas, kidneys, heart, thymus, adrenal, testes, epididymis, ovaries, uterus, cervix, nasal cavity, pharynx, larynx and trachea were subjected to gross pathological evaluations.

2.8 Histopathology

All the animals were scarified at the end of the experimental period. Following organs from the control and test groups were subjected to histopathological evaluations. Organs such as lungs, cerebrum, cerebellum, liver, spleen, pancreas, kidneys, heart, thymus, adrenal, testes, epididymis, ovaries, uterus, cervix, nasal cavity, pharynx, larynx and trachea. All the above organs were collected and preserved in 10% buffered formalin. Blocks of tissue were processed in automatic tissue processor and embedded in molten paraffin. Five micrometer (3–5µm) thick sections were cut from the block and stained with Haematoxylin and Eosin and subjected to microscopical examinations.

2.9 Antioxidant assay

2.9.1Preparation tissue homogenate

Liver homogenate was prepared as follows, liver tissues of Wistar rats exposed to 0.5% of H_2O_2mist at varying doses and control animals were dissected and washed with saline (0.9%) to remove excess blood. The tissues were homogenized in 0.1M phosphate buffer using a tissue homogenizer (Polytron P3100) at 1000 rpm and subsequently centrifuged to pellet cell debris. The supernatant was collected and stored at -80°c until used for antioxidant assays.

2.9.2 Total protein assessment

Total protein concentration of liver homogenate was determined (Lowry et al. 1951) with bovine serum albumin as standard in a UV/Vis spectrophotometer (Perkin Elmer, USA) at 690 nm.

2.9.3 Reduced glutathione level (GSH)

The amount of reduced glutathione (GSH) in the liver homogenate of Wistar rats exposed to 0.5% H_2O_2mist was determined with slight modifications of the method of Moron, Depierre, and Mannervik (Moron et al. 1979). The thiol reagent DTNB (5, 5'-dithiobis- (2-nitrobenzoic acid), reacts with GSH to form a chromophore, 5-thionitrobenzoic acid (TNB) and it is measured using UV/Vis spectrophotometer (Perkin Elmer, USA) at 412 nm.

2.9.4 Glutathione peroxidase (GPx)

GPx activity in liver tissue homogenate of Wistar rats exposed to 0.5% H_2O_2mist was analysed (Rotruck et al.1973). Glutathione peroxidase (GPx) resulting in the formation of glutathione disulfide (GSSG). GSH remaining after enzyme catalyzed reaction complexes with DTNB, which absorbs at maximum wavelength of 412 nm. Enzyme activity was expressed as mg of GSH consumed /min/mg protein.

3. Result

3.1 Skin irritation test

Skin irritation potential on exposure to 0.5% $H_2O_2$was determined and depicted in table 2. Rabbits showed no signs of skin irritation after exposure to 0.5% H_2O_2. The treated skin remained intact, no oedema and erythema compared to control site was observed. Enema and erythema score was categorised (ISO 10993-10) and recorded as '0' in each rabbit on removing the test material and at the end of observation period.

3.2 Inhalation toxicity

3.2.1Clinical signs and survival

No mortality or morbidity was found in both control or tested groups throughout the experimental period. No clinical signs of toxicity were observed on exposure to 0.5% hydrogen peroxide mist in all study groups.

3.2.2 Body weight

Gain in body weight was observed in all groups (low dose, medium dose, high dose and high dose recovery) exposed to 0.5% H_2O_2mist. Significant increase in body weight observed when compared to initial body weight, as shown in Table 3.

3.2.3Haematology

Haematological parameters were analysed separately for males and females. The results (Table 4) revealed that, all the haematological parameters were comparable with the control group, both in males and females. The RBC count and percentage of HCT of control animal was found to be less than the

normal range. It is suggested that the alternation observed in the RBC count or percentage of HCT is not related with the test sample exposure. There is a statistical significance observed in MCV and MCHV values, but the values are within the normal range.

3.2.4 Serum biochemistry

Biochemical analysis revealed serum ALT, bilirubin, creatinine, triglycerides, total protein and albumin in both female and male Wistar rats exposed to0.5% H_2O_2 mist for a period of 7 days were comparable with that of control (Table5a, 5b). However, slight variation was observed in the creatinine, ALT, total protein, albumin and ALP level of control animals in comparison with normal reference range. Significant increase was observed in serum cholesterol and urea levels compared to control in hydrogen peroxide mist exposed Male Wistar rats (P<0.01, P<0.05), but all are within the reference range. Similarly serum glucose levels were also found be increased in Wistar rats (Female) (Table 5a). Moreover, serum AST (SGOT) levels were decreased in both male (high dose) and female Wistar rats (Low, medium, high) with respect to control**.** It was also noted that an increased level of triglycerides occurred in the low and medium dose group of males and high dose group of females. Significant difference was observed in urea, AST, Glucose, and cholesterol levels in recovery group in comparison with control and high dose (p<0.01)

3.2.5 Gross necropsy

Necropsy examination of lungs, cerebrum, cerebellum, liver, spleen, pancreas, kidneys, heart, thymus, adrenal, testes, epididymis, ovaries, uterus, cervix, nasal cavity, pharynx, larynx and trachea in low, medium, high dose and high dose recovery group (both male and female) did not reveal any treatment (0.5% H_2O_2mist) related gross findings in comparison to control group.

3.2.6 Histopathology

Histopathological examination of lungs, cerebrum, cerebellum, liver, spleen, pancreas, kidneys, heart, thymus, adrenal, testes, epididymis, ovaries, uterus and cervix did not revealed any abnormality in low, medium, high dose and high dose recovery group in comparison to control group. Similarly, no pathological lesions were evident in nasal cavity, pharynx, larynx and trachea in low, medium, high dose and high dose recovery group. Histopathology of control and 0.5% of H_2O_2mistexposed tissues of both male and female are shown in Figure 1 (a-f), Figure 2 (a-f) and Figure 3 (A-B).

3.2.7 Antioxidant assay

The results depicted in Table.6 revealed that no significant difference was observed in the levels reduced glutathione and glutathione peroxidase with respect to control.

Statistical analysis

Data were expressed as mean ± SD. Statistical comparisons between and control exposed groups was done using one-way ANOVA followed by Tukey'sposthoc test (SPSS ver 22, IBM Corp) and significant

difference between initial and final body weight by paired T-test. The *p* values ($p < 0.01$, $p < 0.05$) are considered as significant.

5. Discussion

Hydrogen peroxide, its oxidising nature enabled it to be a part of wide range of applications. Among the various applications, both aqueous and gaseous form of H_2O_2 plays a key role in the process of sterilization. Recently the concept of vapour phase H_2O_2 (VPHP) sterilization has been developed as a replacement for EO and formaldehyde sterilants (McEvoy et al. 2019). The rationale of the study is to assess the safety of 0.5% hydrogen peroxide used as a disinfectant compound in the disinfectant gateway developed in concern with current virus pandemic. In the disinfected gate way the hydrogen peroxide mist is used as a disinfectant to decontaminate the personnel entering a cleaner private space from a public space (Krishnan et al. 2020)

In the present study, acute skin irritation and repeated dose inhalation toxicity was carried out to assess safety assessment. Notably, 0.5% of hydrogen peroxide mist was used in disinfectant gateway by exploiting its application as a disinfectant to reduce the microbial and viral load. Skin irritation is considered to be one of the relevant end points of chemicals and therefore skin irritation test is critical, if dermal contact is intended (Chandra et al. 2015). A study on New Zealand rabbits, reported that severity of skin irritation increases within the increase in concentration of hydrogen peroxide solution (10 %< 35 %< 50%). No signs of skin irritation were observed in New Zealand white rabbits administered with 3% aqueous hydrogen peroxide to intact and abraded skin under occlusion for 24 h (ECHA, 2003). In this study, skin irritation test in rabbits was done to screen any potential hazards of hydrogen peroxide on skin and no evidence of erythema and oedema was observed for 0.5% of hydrogen peroxide.

Inhalation of chemicals in many industrial workplaces can be a trigger of respiratory tract disorders as well as systemic diseases, depending on the type of inhaled substance and exposure time (Bakand et al. 2005; Camus et al. 1998). It has been documented that male Wistar rats were exposed to hydrogen vapour (90%) in glass chamber for 8h, sacrificed after 14 days showed alveolar emphysema together with severe congestion (ECHA,2003).A sub-chronic toxicity study on rats exposed to aerosolized hydrogen peroxide (50% aq.) 5 days per week, 6 h per day, for 28 days showed clinical signs such as reddened nose, salivation, irregular breathing, respiratory tract irritation, piloerection, at 14.6 mg/ml (Oberst et al. 1954). In this present repeated dose exposure study, no evidence of mortality, clinical toxicity signs and body weight loss was observed in all study groups compared to control.

Haematology and serum biochemical assessments are key tools to assess the physiological and pathological status of the tissue (Stockham et al. 2003). In this study, the haematological parameters of clinical importance, haemoglobin, white blood cell counts and platelets were observed to be similar to that of control in both female and male. Wistar rats exposed to 0.5% of hydrogen peroxide mist. Alternations were observed in red blood cell (RBC) counts with respect to control, in male Wistar rats exposed to hydrogen peroxide mist (low, medium dose). But it may not be consider as a sign of hydrogen peroxide induced toxicity, since these changes were not observed in high exposure level. However, haematological

indices HCT, MCV, MCHC were significantly decreased in male Wistar rats at low and medium dose exposure of hydrogen peroxide mist and at high dose in females. These variation exist may be due to age, reproductive status, housing, starvation, environmental factors, stress and transportation (Waziri et al. 2010).

The pathological of condition of internal organs after exposure to hydrogen peroxide mist was revealed by serum biochemistry. Variation observed in the creatinine, ALT, total protein, albumin and ALP levels of control animals in comparison with the normal reference range suggested by CPCSEA (CPCSEA, 2003). A significant decrease in serum AST levels was observed in female Wistar rats exposed to hydrogen peroxide (low, high, medium and recovery groups) with respect to control. Whereas in male Wistar rats significant difference was observed at medium and recovery groups with respect to control but all are within the reference range. It was also found that alkaline phosphatase levels (ALP) increased in male Wistar rats at low dose but no such increase was observed at high dose with respect to control. It has been reported that breed, sex, age, reproductive status; time of feeding, diurnal variations, nutritional state and management; and geographical/climatic factors influence serum parameters (Stockham et al. 2013). It was also documented that many of the haematological and biochemical parameters are in association with developmental changes (Lillie et al.1996). So the alternations in serum AST and ALP levels may be due to such variation than the effect of hydrogen peroxide. Significant difference was observed in serum urea and cholesterol levels in male Wistar rats exposed to hydrogen peroxide, in comparison with control but all are within the reference range. In recovery groups, most of the test parameters were found to be comparable with that of control. Nonetheless, significant decreases in urea levels were observed in male Wistar rat. Further, negligible differences observed with regard to haematological and biochemical parameters may not be considered as toxicological or biological significance as the results were comparable to that of control and most of the changes were not related to the exposure.

Histopathological results revealed no abnormalities or pathological alternations in the tissues examined. All the tissues like lungs, cerebrum, cerebellum, liver, spleen, pancreas, kidneys, heart, thymus, adrenal, testes, epididymis, ovaries, uterus, cervix, nasal cavity, pharynx, larynx and trachea examined after H_2O_2 exposure remains intact, similar to that of control. Studies have documented on the morphological complexity and the importance of upper respiratory tract in inhalation toxicity studies (Herbert et al. 2018). Studies have documented that inhalation of chemicals may affect the pulmonary epithelium of the respiratory tract (Gorguner *et al.* 2010).Toxicants or irritant chemicals induce frequent lesions such as necrosis, erosion and ulceration of the respiratory epithelium. In this study, no hydrogen peroxide induced alternations were observed, even though few infrequent lesions observed in control rats, may be due to microbial infections.

Oxidative stress, an imbalance between reactive oxygen species (ROS)and the antioxidant defence mechanism of cells or tissues, lead to rapid glutathione depletion, decreasing antioxidant enzyme, lipid peroxidation and DNA damage (Valko et al. 2016). Antioxidant enzyme system play key role in defence against free radical induced oxidative damage. It has been reported that hydrogen peroxide (100 mg/kg body weight) induced alternations in antioxidant enzyme activity in mice (Yalçın et al. 2020). In normal

conditions the glutathione/glutathione peroxidase system maintains the hydrogen peroxide levels by breaking down to oxygen and water (Reid et al. 2011).In this study no significant difference was observed in reduced glutathione and GPx activity with respect to control.

The results of the present study suggest that, there was no skin irritation potential such as oedema or erythema or necrosis on exposure to 0.5% H_2O_2 in rabbits. Similarly the inhalation toxicity of 0.5% of H_2O_2 mist, intended to be used for disinfection gateway for COVID-19, was well tolerated by wistar rats (both male and female).Further, at the laboratory condition stimulated, the NOEL was found to be 55.11mg/kg body weight. Hence, the present study concluded that 0.5% H_2O_2 or its mist used in the disinfection gateway for COVID-19, failed to induce any skin irritation in rabbits or inhalation toxicity in rats.

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical Approval

Experiments were conducted after obtaining approval from the Institutional animal ethics committee. The animal care and handling was done according to the CPCSEA guidelines for animal experimentation.

Consent to Participate and Publish

All authors read and approved the manuscript and have no conflict in participation and publishing the manuscript.

Authors Contributions

Moahan: design of the study, experiment done, data collection, drafting, interpretation and final approval. Sangeetha, Sabareeswaran, Muraleedharan, Jithin, Vandana, Varsha: data collection, drafting and experimental support.

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Data Availability

The authors declared that the research data referred correctly cited in the manuscript's reference section.

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Tables

Table 1: Experimental design: Repeated dose inhalation toxicity of 0.5% H_2O_2 mist

No	Group	No of animals (Rats)		Duration of exposure	Dose mg/kg body weight of H_2O_2 mist
		Male	Female		
1	Control	4	4	0 min	0
2	Low dose	4	4	2 min	11.022mg/kg
3	Medium dose	4	4	4 min	22.044mg/kg
4	High dose	4	4	10 min	55.11mg/kg
5	High dose recovery	4	4	10 min	55.11mg/kg

Table 2: Skin irritation score of Rabbits exposed to 0.5% H_2O_2

Animal	Group	Irritation score 24h		Irritation score 48h		Irritation score 72h	
1	Control	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
	Test	0	0	0	0	0	0
2	Control	0	0	0	0	0	0
	Test	0	0	0	0	0	0
3	Control	0	0	0	0	0	0
	Test	0	0	0	0	0	0
Primary irritation score		0					
Irritation Index		0					

Table 3: Body weight of Wistar rats before and after exposure to 0.5% H_2O_2 mist

Groups	Initial body weight (g)	Final body weight (g)	Initial body weight (g)	Final body weight (g)
	Male		Female	
Control	242.33 ± 9.54	280.92 ± 11.41**	179.71 ± 6.18	195.72 ± 2.93*
Low dose	171.50 ± 13.82	263.02 ± 7.76**	171.82 ± 11.50	199.80 ± 7.74*
Medium dose	233.11 ± 10.99	262.15 ± 18.14**	183.55 ± 9.33	192.26 ± 5.65
High dose	217.28 ± 33.86	252.53 ± 45.53**	183.96 ± 9.28	190.31 ± 11.94
High recovery$ dose	195.50 ± 18.53	285.00 ± 26.52**	176.70 ± 5.80	200.80 ± 10.17**

Data expressed as mean ± SD. Statistical significance was determined using paired T-test. **$p< 0.01$; *$p< 0.05$ compared to initial body weight.(Final body weight after 7 days of exposure), $Final bodyweight after14days

Table 4: Haematological values of rats exposed to 0.5% H_2O_2 mist

Groups	Hb (g/dl)	RBC(x 10^6/mm^3)	WBC (x10^3/mm^3)	PLT(x10^3/mm^3)	HCT (%)	MCV (um^3)	MCHC (g/dL)
Male							
Control	15.07± 0.58	5.23± 0.19	10.00± 2.80	836.3± 41.30	34.20± 1.12	65.30± 1.71	44.13± 0.61
Low dose	15.10± 0.49	5.98± 0.15*	8.40± 2.30	815.3± 60.11	37.10± 1.36*	61.80± 2.50*	40.60± 0.24**
Medium dose	15.63± 0.79	6.00± 0.22*	8.15± 1.90	847.5± 26.30	37.53± 1.94*	61.30± 1.50**	41.70± 0.58**
High dose	14.50± 0.61	4.85± 0.12	8.68± 1.40	848.5± 22.65	32.03± 1.68	66.00± 1.63	45.30± 0.77
High dose Recovery	15.00± 0.64	6.20± 0.19**#	10.50± 4.48	888.7± 19.63	38. 90± 2.97**#	59.80± 1.50**#	37.88± 1.46**#
Female							
Control	14.65± 0.43	4.85± 0.05	6.35± 2.70	824.5± 66.54	31.78± 0.54	65.30± 0.50	45.58± 0.85
Low dose	14.70± 0.82	5.13± 0.17	6.18± 1.29	776.3± 50.26	32.45± 1.76	63.30± 2.06*	45.35± 0.85
Medium dose	14.18± 0.45	4.85± 0.21	6.02 ± 0.58	862.0± 18.13	31.23± 1.41	64.80± 0.50	45.33± 0.84
High dose	14.65± 0.17	5.68± 0.22**	6.03± 0.59	852.3± 35.03	33.93± 1.38	60.00± 0.50**	43.25± 1.09*
High dose Recovery	15.30± 0.49	6.74± 0.44**#	6.53± 0.55	888.3± 20.02	39.20± 2.18**#	58.00± 0.82**#	38.98± 0.87**#

Data expressed as mean ± SD, n = 4. Statistical significance was determined using one way ANOVA followed by Tukeys post hoc test.*$p < 0.05$; **$p < 0.01$ compared to control, #$p < 0.01$ compared to high dose

Table 5a: Serum biochemistry of rats exposed to 0.5% H_2O_2 mist

Groups	Urea Mg/dL	Creatinine (mg/dL)	SGPT (ALT) (IU/L)	SGOT (IU/L)	Glucose (mg/dL)
Male					
Control	43.17± 1.50	0.90± 0.19	83.43± 4.48	215.9± 26..89	77.10± 10.27
Low dose	38.79± 3.50*	0.89± 0.02	66.55± 33.64	199.0± 4.32	77.20± 11.43
Medium dose	38.10± 1.00*	0.62± 0.18	64.40± 18.15	153.5± 41.8*	79.38± 10.81
High dose	39.23± 3.96	1.00± 0.14	66.83± 19.28	176.3± 55.57	56.32± 7.40*
High dose recovery	15.32± 2.25**#	1.03± 0.20	71.78± 4.80	128.4± 12.14**#	97.40± 9.00**#
Female					
Control	40.63± 1.07	0.94± 0.12	81.38± 18.76	221.4± 27.7	48.38± 3.12
Low dose	31.43± 5.20*	1.00± 0.12	82.93± 25.29	170.7± 47.78*	44.60± 19.46
Medium dose	37.34± 1.35	1.10± 0.12	69.93± 18.62	173.5± 17.28*	53.10± 18.88*
High dose	38.56± 0.33	1.10± 0.12	65.38± 6.29	152.2± 54.39**	76.40± 8.63**
High dose recovery	23.35± 5.68**#	0.91± 0.11	66.05± 7.53	134.2± 5.96**	82.63± 11.87**#

Data expressed as mean ± SD, n = 4. Statistical significance was determined using one way ANOVA followed by Turkey'spost hoc test. **p< 0.01; *p < 0.05 compared to control,#p< 0.01 compared to high dose

Table 5b: Serum biochemistry of rats exposed to 0.5% H_2O_2 mist

Groups	Cholesterol (mg/dL)	TG mg/dL	ALP IU/L	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin g/dL
Male						
Control	112.3± 8.73	155.8± 3.30	227.0± 103.6	0.25± 0.09	11.07± 4.18	5.30± 1.17
Low dose	73.50± 13.00*	198.8± 26.74	336.0± 90.00*	0.25± 0.05	9.45± 1.60	5.15± 0.26
Medium dose	81.20± 8.75*	185.5± 84.86	336.2± 84.26	0.27± 0.09	10.80± 0.59	5.33± 0.48
High dose	89.00± 38.9	154.8± 48.53	364.1± 7.66	0.28± 0.08	10.87± 2.43	5.50± 1.00
High dose recovery	61.41± 5.56**#	120.3± 21.09	185.9± 68.20#	0.34± 0.85	9.90± 1.62	5.07± 0.26
Female						
Control	80.0± 11.92	100.0± 8.04	181.3± 16.70	0.29± 0.09	10.75± 2.90	5.63± 1.07
Low dose	90.3± 22.4	101.5± 27.74	186.3± 18.15	0.34± 0.34	11.90± 2.28	4.98± 0.56
Medium dose	84.08± 6.08	150.0± 58.09	190.8± 28.8	0.33± 0.07	10.63± 0.62	5.40± 0.91
High dose	78.25± 26.72	214.0± 58.09**	203.0± 82.32	0.33± 0.09	11.85± 0.94	5.88± 0.45
High dose recovery	56.40± 5.6**#	102.3± 18.25#	110.6± 39.43	0.37± 0.05	10.40± 2.84	5.50± 0.43

Data expressed as mean ± SD, n = 4. Statistical significance was determined using one way ANOVA followed by Tukeys post hoc test. **p< 0.01; *p < 0.05 compared to control, #p< 0.01 compared to high dose

Table 6: Effect of antioxidant enzymes on exposure of 0.5% H_2O_2 mist to male wistarrats

Groups	Reduced GSH (nmol/mg protein)	GPx (units/mg protein)
Control	1.03 ± 0.02	0.11 ± 0.60
Low	1.04 ± 0.29	0.11 ± 0.91
Medium	1.10 ± 0.25	0.12 ± 0.92
High	0.87 ± 0.03	0.20 ± 0.17
High recovery	0.90 ± 0.13	0.24 ± 0.043

Data expressed as mean ± SD.Gpx : Glutathione peroxidase, GSH: Reduced glutathione

Figures

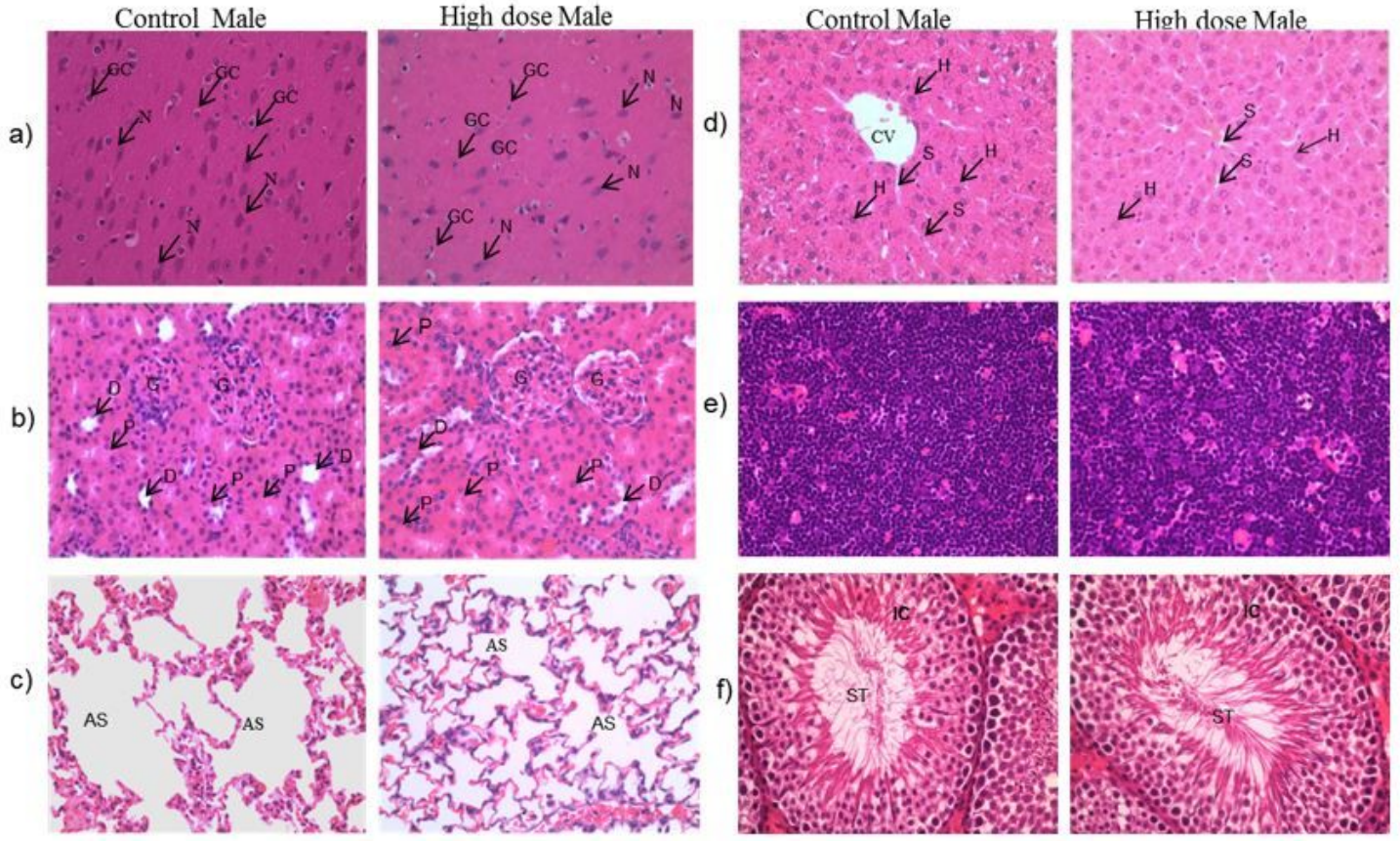

Figure 1

Histopathology of rats (male) exposed to 0.5% H2O2 mist: Brain (a): Neuron (N), Glial cells(GC); Kidney(b): Glomeruli (G), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT); Lung (c): Alveolar space (AS); Liver (d): Central vein (CV), Hepatocyte (H), Sinosoids (S); Testis (f): Seminiferous tubule (SF). Magnification (400x).

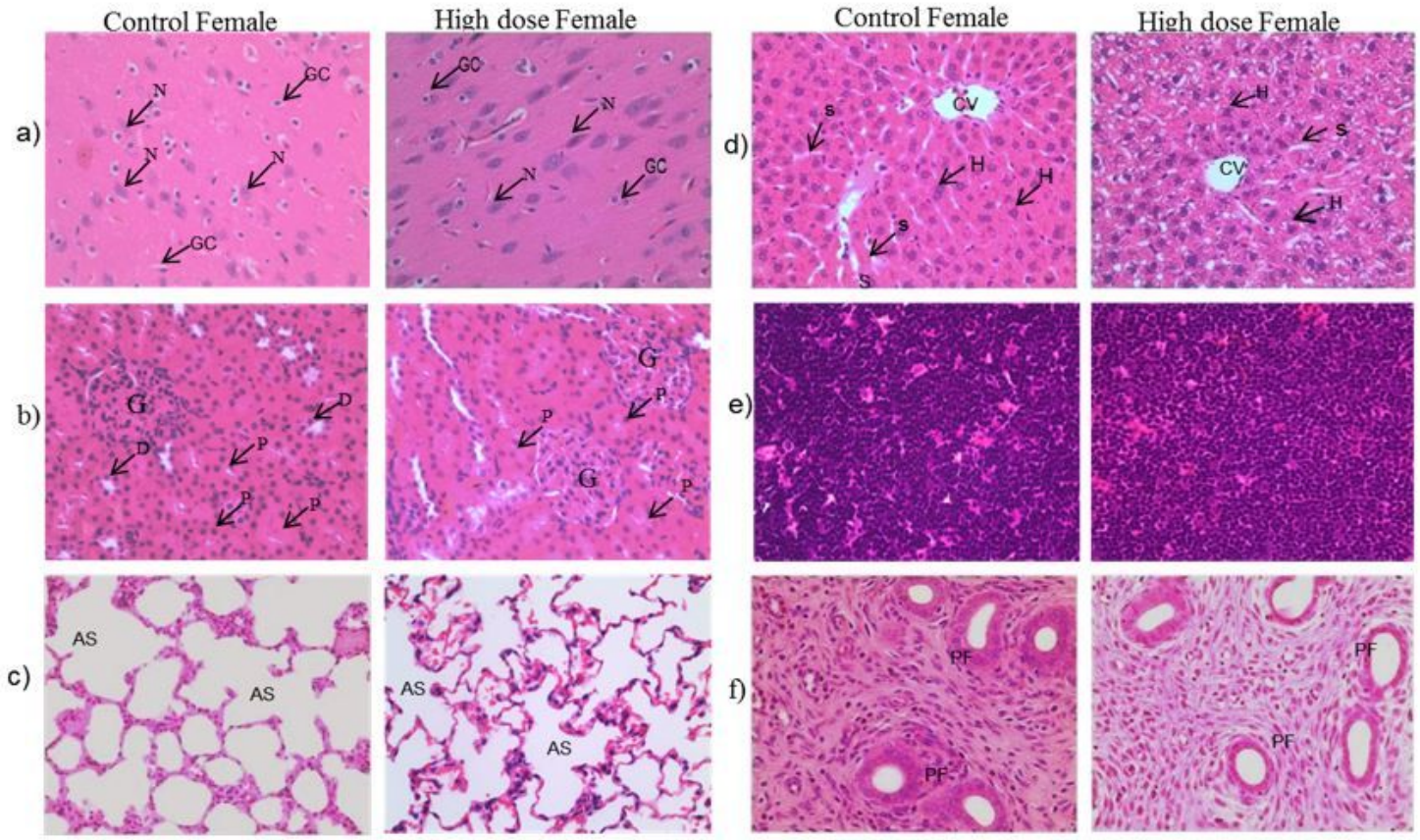

Figure 2

Histopathology of rats (female) exposed to 0.5% H2O2 mist: Brain (a): Neuron (N), Glial cells (GC); Kidney (b): Glomeruli (G), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT); Lung (c): Alveolar space (AS); Liver (d): Central vein (CV), Hepatocyte (H), Sinosoids (S); Ovary (f): Primary follicle (PF).Magnification:400x.

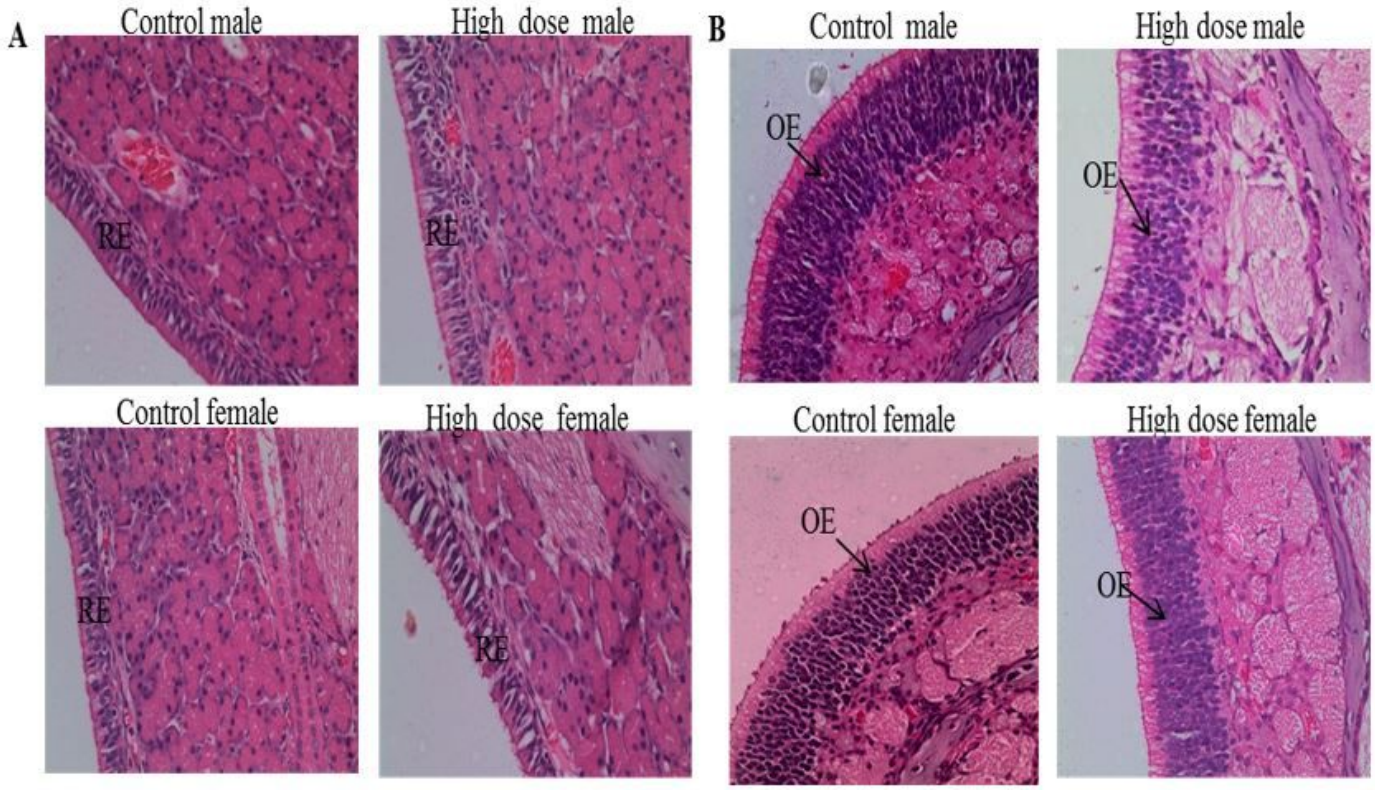

Figure 3

Histopathology of rats (male and female) exposed to 0.5% H2O2 mist: (A) Respiratory epithelial, (B) Olfactory epithelial areas of the nasal cavity. RE: Respiratory epithelium, OE: Olfactory epithelium. Magnification: 400x

Supplementary Files

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