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Research Article

Formaldehyde Exposure Induces Bronchial, Haematological, and Biochemical Alterations in Wistar Rats

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ABSTRACT

Background: Formaldehyde fumes are hazardous environmental pollutants with established respiratory toxicity; however, their effects on bronchial histoarchitecture alongside systemic haematological and biochemical changes remain incompletely characterized.

Methods: This randomized controlled experimental study involved twenty-four adult Wistar rats of both sexes (248–260 g), assigned into four groups (n = 6). Group A served as control, while Groups B, C, and D were exposed to increasing concentrations of formaldehyde fumes generated from 10ml, 20ml, and 40ml of 40% formaldehyde, respectively, for 28 days. Bronchial histology, haematological indices, and biochemical parameters, including serum bicarbonate, urea, and creatinine, were assessed. Data were analyzed using one-way ANOVA at $p < 0.05$.

Results: Formaldehyde exposure produced concentration-dependent alterations across all evaluated parameters. Serum bicarbonate levels showed a significant, dose-dependent decline in formaldehyde-exposed groups compared with the control group (A) ($P < 0.05$), indicating disturbance in acid–base balance. In contrast, serum urea and creatinine levels increased significantly in exposed groups relative to controls ($P < 0.05$), reflecting progressive impairment of renal function with increasing formaldehyde exposure.

Haematological analysis revealed significant reductions in lymphocytes, basophils, red blood cell indices, haemoglobin, and haematocrit, alongside elevated reticulocyte counts ($p<0.05$ each), suggesting anaemia and immune suppression. Histological examination of bronchial tissues demonstrated dose-dependent pathological changes, including mucosal ulceration, inflammatory cell infiltration, activated lymphoid follicles, and luminal haemorrhage, consistent with bronchitis.

Conclusion: Sub-chronic inhalation of formaldehyde fumes induces dose-dependent bronchial histopathology, haematological dysregulation, electrolyte imbalance, and renal dysfunction in Wistar rats. These findings underscore significant occupational health risks and highlight the need for stringent exposure control measures.

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1. Introduction

Formaldehyde, a colorless and highly volatile liquid, is utilized in various industries, including healthcare, research, and manufacturing (Zendehdel *et al*., 2016; Sahu *et al*., 2022). Despite its widespread use, formaldehyde exposure has been recognized as a significant occupational hazard, particularly for workers handling formaldehyde-based products or equipment (Wand & Christiani, 2000; Ahmad *et al*., 2013; Ehi-Omosun *et al*., 2025;). Recent studies have shown that formaldehyde exposure induces oxidative stress, airway inflammation, and systemic physiological changes, including haematological and renal dysfunction in animal models (Abd-Elhakim *et al*., 2016; Murta *et al*., 2016; De Oliveira Rames *et al*., 2017).

Furthermore, formaldehyde exposure can trigger the release of pro-inflammatory mediators such as interleukin-6, leukotriene B4 and bradykinin, leading to inflammation and damage to bronchial tissue (WHO,1989; Poinen *et al*., 2016). This can contribute to the development of respiratory diseases, such as bronchitis, bronchiectasis, and asthma. Furthermore, studies have demonstrated that formaldehyde exposure can cause respiratory allergy, shortness of breath, and other respiratory illnesses in experimental animals (Fischer, 1905; George *et al*., 2014; Protano *et al*., 2021).

The impact of formaldehyde exposure on haematological parameters is commonly evaluated by examining a range of blood markers, including white blood cell count, lymphocyte subset analysis, platelet count, haemoglobin levels, haematocrit, and red blood cell indices (Cheesbrough, 2005; Balogun *et al*., 2014; Szyszlak-Barglowicz *et al*., 2022). Biochemical consequences, on the other hand, are assessed by analyzing parameters like bicarbonate, urea, and creatinine (Fatima *et al*., 2001; Fell *et al*., 2003; Garcia-Perez *et al*., 2015).

The bronchi are a vital part of the respiratory system, serving as the airways that transport air from the trachea to the lungs (El-hilaly *et al*., 2004; Drake *et al*., 2009; Eteng *et al*., 2018). They are branching tubes that divide into smaller and smaller airways, eventually leading to the alveoli in the lungs (Ganapathy *et al*., 2019; Natalini *et al*., 2023). The bronchi begin at the trachea, which splits into two primary bronchi, one for each lung (Drake *et al*., 2009). These primary bronchi then divide into secondary bronchi, which supply air to the different lobes of the lungs (Pronk *et al*., 2006; Robberts *et al*., 2021). The secondary bronchi further subdivide into tertiary bronchi, and then into smaller bronchioles, which eventually lead to the alveoli (Koyama *et al*., 1994; Noh & Hong, 2001).

The walls of the bronchi are lined with a layer of epithelial cells, which produce mucus to trap dust, bacteria, and other foreign particles that enter the airways (Empey, 1978; Omigie *et al*., 2024). The epithelial cells also contain cilia, which are tiny hair-like structures that help to move mucus and debris upwards towards the throat, where it can be coughed out (Lopez-Vidrieio and Rud, 1978; Eapen *et al*., 2017). The bronchi also contain smooth muscle, which allows them to constrict or dilate in response to different stimuli (Pemberton and Goldberg, 1954; Trulock, 1997). This ability to change diameter is important for regulating airflow and responding to changes in the respiratory system (Ghosh *et al*., 2013; Chen *et al*., 2013). In addition to their role in conducting air, the bronchi also play a critical role in the immune system. They contain immune cells, such as macrophages, basophils, and lymphocytes, which help to defend the body against infection and disease (Janson *et al*., 2001; Paradis *et al*., 2016). Their proper functioning is essential for maintaining good health.

The respiratory tract, specifically the bronchi, is susceptible to damage from formaldehyde exposure, leading to conditions such as bronchitis, asthma, chronic obstructive pulmonary disease (COPD), bronchomalacia and bronchiectasis (Porter and Kaplan, 2011). Chronic bronchitis, often caused by occupational exposures, increases the risk of developing bronchiolitis (Yang *et al*., 2005; Gizaw *et al*., 2016). Formaldehyde exposure has been linked to respiratory diseases, including bronchitis and bronchiectasis, characterized by inflammation and damage to the bronchi (Krzyzavowski *et al*., 1990; Porter and Kaplan, 2011). While existing literature has predominantly focused on systemic haematological and biochemical outcomes of formaldehyde exposure, studies that integrate detailed bronchial histological evaluation under controlled, dose-dependent conditions are scarce. This histological component constitutes an original contribution of the present study.

Although previous research has documented the respiratory and systemic effects of formaldehyde exposure, there is a lack of comprehensive studies that concurrently assess bronchial histological alterations alongside detailed haematological and biochemical profiles under graded exposure conditions. Therefore, this study aimed to investigate the dose-dependent effects of formaldehyde fume inhalation on bronchial histoarchitecture, systemic haematological indices (including white blood cell differentials and red blood cell indices), and key biochemical markers (serum bicarbonate, urea, and creatinine) in Wistar rats.

2. Materials and Methods

2.1 Experimental Animals

Twenty-four adult male and female Wistar rats (248–260 g) were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria. The animals were housed in groups of six per cage, with wood shavings as bedding, under controlled environmental conditions. The housing conditions were designed to minimize stress and ensure animal comfort. The animals were acclimatized for two weeks before the experiment began. During this period, they were accustomed to handling, had access to standard animal feed and clean water *ad libitum.*

2.2 Ethical Considerations

Ethical approval for this study was obtained from the Research Ethical Committee of the College of Medical Sciences, University of Benin, Nigeria (CMS/REC/2024/098). Animal procedures were performed in compliance with approved protocols and in accordance with the recommended guidelines for the care and use of laboratory animals (Buzek and Chastel, 2010).

2.3 Experimental design

This study employed a randomized block design, dividing 24 rats into four groups. The groups were matched based on age, sex, and body weight, and a blind procedure was implemented for group assessment and treatment administration. The rats were exposed to graded levels of formaldehyde fumes generated from different volumes (0 ml, 10 ml, 20 ml, and 40 ml) of 40% formaldehyde solution for 28 consecutive days using a formaldehyde fume-free fume distributor glass chamber (FDGC). Formaldehyde exposure was carried out in a fume distributor glass chamber (FDGC) maintained under controlled laboratory conditions. The chamber temperature was kept at 25 ± 2 °C, with relative humidity maintained at 50–60% throughout the exposure period. These conditions were monitored daily to ensure uniform exposure and minimize environmental variability. The dosages were determined based on a thorough review of existing literature on toxicological studies involving formaldehyde exposure in animal models. Our selection of these dosages was also guided by the results of our pilot study, which employed a sample of 5 animals, with 1 animal per group. This pilot study suggested that these dosages were well-tolerated by the rats.

2.4 Method of Termination and Sample Collection

On the 28th day of exposure to formaldehyde, the animals were euthanized under chloroform anesthesia. Blood samples were collected through cardiac puncture into anticoagulant (Ethylene Diamine Tetra acetic Acid) bottles for haematological analysis and into plain specimen bottles for biochemical analysis. The lung of each rat was excised and fixed in 10% formal saline for 24 hours before the histological procedures. The tissues were trimmed to about 5 micrometer thick sections and processed according to the method of Carleton *et al*., 1967. The trimmed tissues underwent manual histological processing for microscopy involving fixation in 10% neutral buffered formalin (pH 7.4) for 24-48 hours at room temperature, followed by embedding in paraffin wax using a Leica TP1020 tissue processor at 58 ^{0}C, 100 mbar, and 12 hours. Subsequently, tissues were stained with Hematoxylin and Eosin (H&E) using a Thermo Scientific Gemini tissue stainer at 37 ^{0}C for 30 minutes. This H&E staining enabled visualization of tissue morphology and architecture, allowing for the evaluation of histological changes and their correlation with experimental findings. Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at magnification of x40.

A comprehensive haematological analysis was performed using an auto-analyzer (Hoddlier and Stoughton, London, UK) to measure white blood cell count, lymphocytes, monocytes, neutrophils, eosinophils, basophils, red blood cells, haemoglobin, hematocrit, and platelets. Electrolyte analysis was conducted on a Roche Diagnostics Electrolyte Analyzer (Roche Diagnostic Group of Company, Germany). Serum electrolytes were measured using ion-selective potentiometry, while blood urea and creatinine levels were determined using the hypobromite method and calorimetry method, respectively.

2.5 Statistical analysis

Data were analyzed using Microsoft Excel (2010) and the Statistical Package for Social Sciences (SPSS), version 20.0 (IBM Corp., Armonk, NY, USA). Results were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to compare mean values among groups, and differences were considered statistically significant at $P < 0.05$.

All abbreviations used in this manuscript are defined at first mention and applied consistently throughout the text, tables, and figures.

3. Results

3.1 Biochemical Test Findings

The biochemical findings are presented in Figures 1–3.

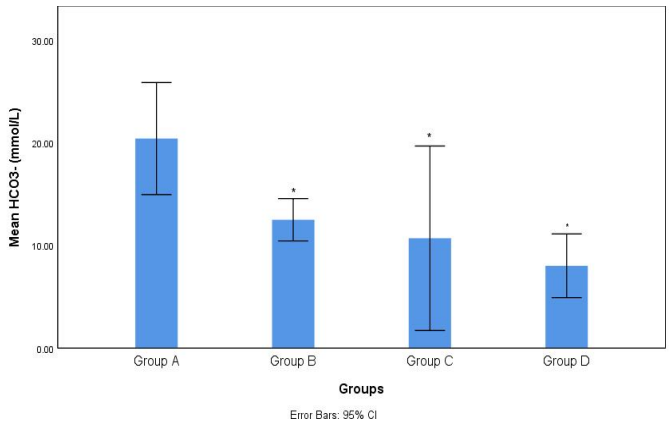


Figure 1: Serum Level of bicarbonate (mmol/L) in the unexposed and exposed groups. The bicarbonate level in the exposed groups (Group B, C and D) when compared with the control group (Group A), showed significant decrease (P<0.05).

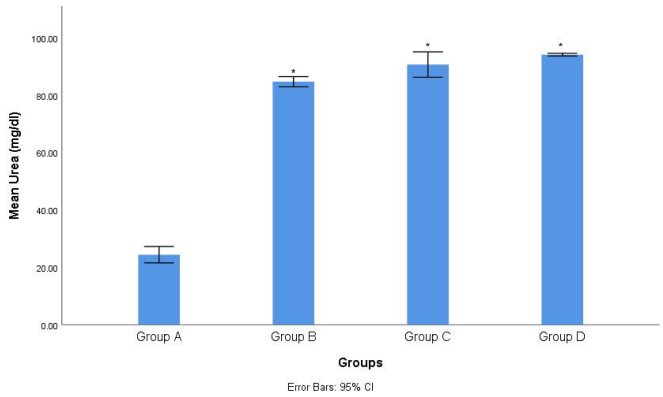


Figure 2: Serum level of urea (mg/dl) in the unexposed and exposed groups *P<0.05 vs control. Blood Urea level in the exposed Group B, C and D when compared with the control (Group A) showed significant increase (P < 0.05).

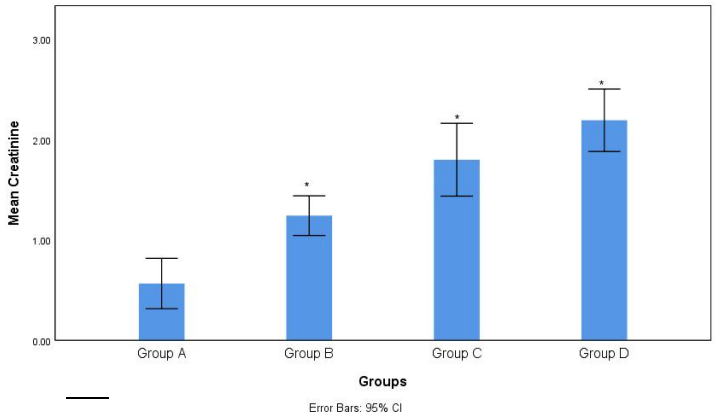


Figure 3: Serum level of creatinine (mg/dl) in the unexposed and exposed groups. Creatinine level in the exposed Group B, C and D when compared with the control (Group A) shows significant increase (P < 0.05).

Serum bicarbonate levels (Figure 1) showed a significant, dose-dependent decline in formaldehyde-exposed groups (B–D) compared with the control group (A) (P < 0.05), indicating disturbance in acid–base balance. In contrast, serum urea (Figure 2) and creatinine levels (Figure 3) increased significantly in exposed groups relative to controls (P < 0.05), reflecting progressive impairment of renal function with increasing formaldehyde exposure.

3.2 Haematological Test Findings

Haematological analysis revealed that formaldehyde exposure predominantly affected the myeloid lineage, particularly red blood cell indices. Significant reductions were observed in red blood cell count, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration, alongside a significant increase in reticulocyte count (P < 0.05). Basophil counts also declined significantly, whereas total white blood cell counts and other leukocyte subsets showed no statistically significant changes.

The haematological findings are presented in Table 1.

Table 1: Comparison of Haematological Parameters In all the Experimental Groups

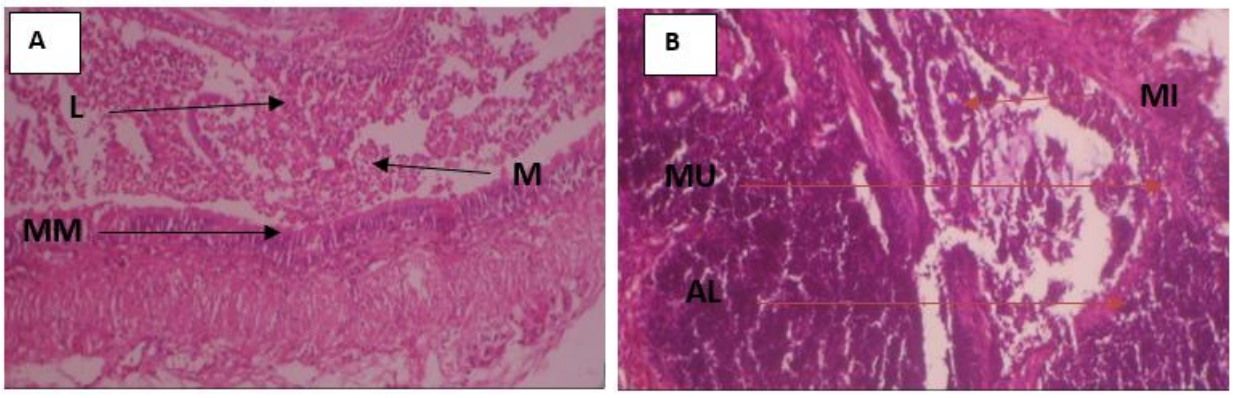
Haematological Parameters	Groups				P- Values
	A	B	C	D	
	(Control)	(10ml of HCHO)	(20ml of HCHO)	(40ml of HCHO)	
LYM (%)	78.80 ± 1.06	74.06 ± 3.64	69.04 ± 6.42	58.18 ± 2.06*	0.007
WBC (10^3/uL)	12.08 ± 3.02	11.46 ± 2.28	10.66 ± 0.88	9.22 ± 1.65	0.800
MON (%)	25.84 ± 6.70	20.50 ± 1.38	19.40 ± 1.74	17.94 ± 2.09	0.473
NEU (%)	9.64 ± 0.98	8.92 ± 1.02	7.20 ± 1.28	6.34 ± 0.95	0.152
EOS (%)	1.84 ± 1.35	1.34 ± 0.56	1.02 ± 0.46	0.69 ± 0.27	0.718
BAS (%)	9.46 ± 1.12	6.10 ± 1.80	4.68 ± 0.76	3.90 ± 0.56*	0.016
RBC (10^6/ul)	10.12 ± 0.70	9.10 ± 0.28	8.88 ± 0.24	7.02 ± 0.18*	0.001
HGB (g/dl)	13.02 ± 0.72	12.10 ± 0.32	11.12 ± 0.38*	10.66 ± 0.22*	0.012
HCT (%)	43.28 ± 1.64	42.54 ± 2.08	38.36 ± 1.36	36.52 ± 1.44*	0.010
MCV (Fl)	45.58 ± 0.40	45.30 ± 0.61	44.32 ± 0.30*	42.94 ± 0.48*	0.000
MCH (pg)	17.12 ± 0.20	16.88 ± 0.26	16.32 ± 0.16	15.10 ± 0.26*	0.000
MCHC (%)	35.80 ± 0.28	36.04 ± 0.30	34.96 ± 0.26	34.04 ± 0.00*	0.004
RET (%)	0.14 ± 0.04	0.52 ± 0.12*	0.96±0.30*	1.60±0.74*	0.000
PLT (10^3/uL)	814.00 ± 17.78	804.60 ± 58.08	796.40 ± 5.82	740.18 ± 52.48	0.674

Values are Mean ± S.E.M *P<0.05 vs. control

Key: Formaldehyde (HCHO), Lymphocytes (LYM), White blood cells (WBC), Monocytes (MON), Neutrophils (NEU), Eosinophils (EOS), Basophils (BAS), Red blood cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean cell volume (MCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Reticulocytes (RET), Platelets (PLT).

3.3 Histological Findings

The study's histological examination revealed significant changes in exposed groups. Formaldehyde fumes caused mucosal ulceration, inflammatory cell infiltration, and activated lymphoid follicles (Figure 4A-D). The severity of these changes increased with formaldehyde concentration, with severe mucosal ulceration and luminal haemorrhage observed in groups exposed to higher concentrations. Representative photomicrographs of bronchial sections are shown in Figure 4.



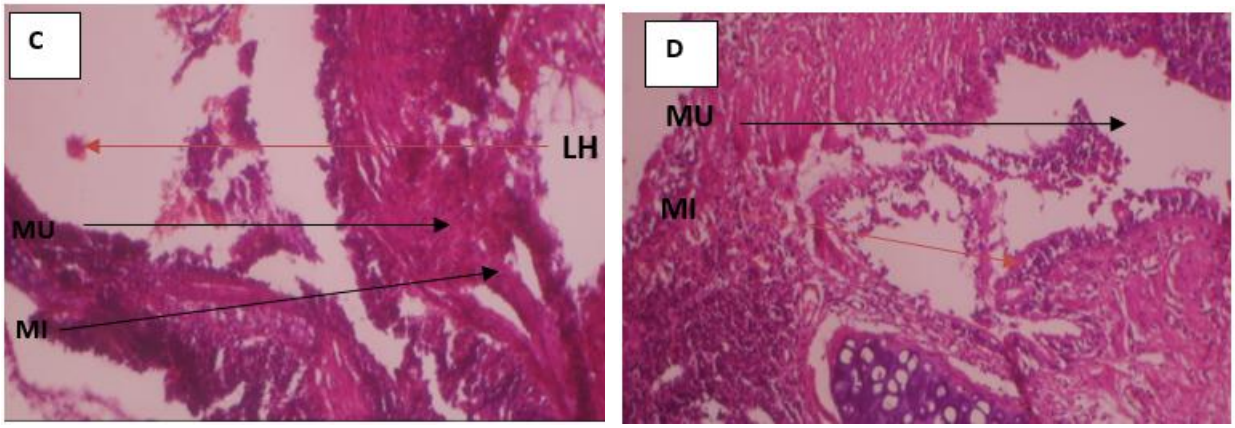


Figure 4: Representative high-resolution photomicrographs of H&E-stained bronchial sections at ×40 magnification.
(A) Control group showing normal bronchial lumen (L), mucosal membrane (MM), and muscularis (M)
(B) Group B (10 mL formaldehyde exposure) showing inflammatory cell infiltration (MI), activated lymphoid follicles (AL), and mucosal ulceration (MU).
(C) Group C (20 mL exposure) showing severe inflammatory infiltration, marked mucosal ulceration, and luminal haemorrhage (LH).
(D) Group D (40 mL exposure) showing extensive inflammatory infiltration and severe mucosal ulceration.

4. Discussion

The inhalation of formaldehyde fumes is associated with an increased risk of respiratory diseases and other health issues in humans and animals, but a comprehensive understanding of the specific impacts on the bronchi is still lacking. This study investigates the toxicological effects of formaldehyde fume exposure on bronchial tissue histology, haematological profiles, and biochemical parameters in Wistar rats. The findings of this study demonstrate that sub-chronic inhalation of formaldehyde fumes exerts marked toxic effects on bronchial structure and systemic physiology. The observed bronchial mucosal injury, haematological dysregulation, and biochemical disturbances highlight the vulnerability of the respiratory tract and vital organs to inhaled formaldehyde. These results underscore significant occupational and environmental health implications, particularly for individuals with prolonged or repeated exposure, and emphasize the need for stricter exposure control and monitoring.

Biochemical analysis revealed significant alterations in serum urea, creatinine, and bicarbonate levels in formaldehyde-exposed groups (Figures 1-3). Bicarbonate (HCO_3^-) levels declined significantly ($p<0.05$) in groups B-D relative to the control group (Group A; Figure 1), indicating a potential disturbance in acid-base balance which can lead to various diseases and complications e.g., respiratory acidosis, metabolic acidosis, respiratory alkalosis, metabolic alkalosis and osteoporosis (Porter and Kaplan, 2011). The observed increased levels of urea and creatinine in the exposed groups (Figures 2 and 3) indicate uremia, aligning with previous research by Bello *et al.*, (2015). These biochemical derangements indicate significant renal and acid–base disturbances that may predispose exposed individuals to adverse health outcomes.

The study's haematological findings revealed that formaldehyde fume exposure disrupts various blood parameters, including lymphocytes, basophils, haemoglobin, and haematocrit (Table 1). Notably, exposed groups exhibited decreased lymphocyte and basophil counts, implying impaired immune function and increased susceptibility to infections. Moreover, exposed animals displayed reduced red blood cell parameters, leading to microcytic normochromic anaemia, consistent with Zenebuoya *et al*. (2016) findings. Decreased haemoglobin and haematocrit levels, accompanied by increased reticulocyte counts (Table 2), indicated an effective erythropoietic response (Elgailani and Alsakka, 2016).

Inhalation of formaldehyde fumes induced a systemic inflammatory response (Figure 4B-D) leading to bronchial toxicity, which likely compromised overall health and contributed to reduced immune cells. This finding is consistent with Abuzant *et al.* (2017), who suggested that reduced lymphocytes, basophils, and other immune cell counts in formaldehyde-exposed rats indicates the toxicological effects of its chemical constituents. Histological analysis of bronchial tissues from groups B, C, and D showed consistent histopathological changes, including mucosal ulceration, inflammatory cell infiltration, activated lymphoid follicles, and luminal haemorrhage (Figure 4B-D). These lesions may lead to severe respiratory complications, such as chest pain, bronchitis, and impaired lung function. Moreover, luminal hemorrhage

can cause impaired gas exchange, leading to hypercapnia, pulmonary hypertension, and hypoxemia, potentially resulting in organ failure. The histopathological findings of this study suggest that exposure to formaldehyde fumes can have severe health consequences, including formaldehyde poisoning, hypoxemic respiratory failure, airway hyperresponsiveness, chronic bronchitis, and bronchiectasis. Given the similarities in biological responses between rats and humans, it is reasonable to conclude that these findings have significant implications for human health, emphasizing the need for protective measures to mitigate the risks associated with occupational exposure to formaldehyde fumes.

This study provides critical insights into the adverse effects of formaldehyde fumes on respiratory and overall health. To prevent and manage related health hazards, a multifaceted strategy is necessary. This includes implementing safety protocols, conducting regular medical check-ups for individuals in formaldehyde-related occupations, and promoting public awareness through information campaigns. Additionally, adopting modern technologies in formaldehyde factories can reduce environmental emissions and minimize risks.

5. Study Limitations

Despite the strengths of this study, certain limitations should be acknowledged. The sample size was relatively small, which may limit the generalizability of the findings. In addition, mechanistic assays assessing oxidative stress, inflammatory mediators, or molecular pathways were not conducted. Future studies incorporating larger sample sizes and mechanistic analyses would provide deeper insight into the pathways underlying formaldehyde-induced bronchial and systemic toxicity.

6. Conclusion

This study provides unequivocal evidence that formaldehyde fume exposure has detrimental effects on the bronchi, and overall respiratory well-being in Wistar rats, leading to histomorphological alterations, and haematological and biochemical disruptions. These results emphasize the importance of implementing protective measures and regular health monitoring for individuals occupationally exposed to formaldehyde fumes, especially in industrial settings.

7. Recommendations

To minimize the risks associated with formaldehyde exposure, we recommend implementing robust safety protocols, including the provision of personal protective equipment. Regular medical check-ups for factory workers can facilitate early detection of potential health issues. Public awareness campaigns can educate communities about the risks and prevention strategies, while investing in modern machinery and technologies can significantly reduce environmental formaldehyde emissions.

8. Conflict of Interest

The authors declare that there are no conflicts of interest associated with this study.

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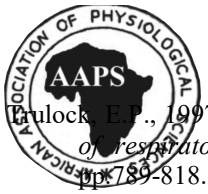

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