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# Ameliorative Effects of *Cymbopogon citratus* Aqueous Leaf Extract on Dichlorvos–induced Hypersensitivity Pneumonitis in Wistar Rats

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## ABSTRACT

Occupational dichlorvos exposure can cause organophosphate poisoning, which can be fatal if left untreated. This study aimed to investigate the effects of aqueous leaf extract of *Cymbopogon citratus* on dichlorvos-induced hypersensitivity pneumonitis in Wistar rats. Thirty Wistar rats weighing between 236 and 256 g were divided into five groups of six rats each. Group A rats were administered feed and water only. Group B rats were exposed to 2 puffs of dichlorvos. Group C rats received 500 mg/kg body weight per day (BWT/D) of *Cymbopogon citratus* only. Groups D and E rats were exposed to 2 puffs of dichlorvos and received 250 mg/kg BWT/D and 500 mg/kg BWT/D of *Cymbopogon citratus* respectively. The dosages of the extract were given for 30 days via an orogastric tube. At the end of the 30th day of exposure, the animals were euthanized under chloroform anaesthesia and the lungs were harvested and processed for histological examination. The histological sections of the lungs of rats in Groups A, C, D and E showed normal histoarchitecture of the lungs. There were observable histological variations in the lung histoarchitecture of the rats exposed to dichlorvos (Group B) which include alveolar hemorrhage, bronchiolar hemorrhage, and interstitial infiltrates of inflammatory cells (evidence of hypersensitivity pneumonitis) This study demonstrates that *Cymbopogon citratus* has a protective role in combating dichlorvos-induced hypersensitivity pneumonitis, characterized by reduced severity of bronchiolar and alveolar hemorrhage and interstitial inflammation, in Wistar rats.

**Keywords:** *Cymbopogon citratus*; Dichlorvos; Histoarchitecture; Hypersensitivity pneumonitis; Exterminators

## INTRODUCTION

Dichlorvos is a chemical compound belonging to the organophosphate family. It is widely used as an insecticide to control household pests, in public health, and protect stored products from insects[1]. Pest eradicators, exterminators and pest control technicians are often exposed to dichlorvos daily for hours during their work. Breathing the fumes of dichlorvos insecticide can occur during fumigation while working directly with dichlorvos or using equipment sprayed with dichlorvos[2]. Dichlorvos contains an organic chemical, dimethoate-cyfluthrin, the active ingredient which is toxic to humans with significant morbidity and mortality if left untreated[3]. Inhalation of these dimethoate-cyflutrinas fumes can cause hypersensitivity pneumonitis and other respiratory problems[3]. Moreover, dichlorvos can trigger inflammation in the lungs, airways, and other tissues, exacerbating conditions like asthma and chronic obstructive pulmonary disease[4].

Previous studies have shown that exposure to dichlorvos fumes causes respiratory allergy and shortness of breath in experimental animals[5]. Dichlorvos vapour, a severe respiratory and skin irritant exerts its toxicity by inhibiting acetylcholinesterase, leading to acetylcholine

accumulation, hyperstimulation, and disruption of neural function, causing neurotoxicity, respiratory failure, and metabolic perturbations[6]. This results in a range of symptoms, from coughing, sneezing, tachypnea, pulmonary oedema, chest pain, and muscle weakness to paralysis and respiratory distress which can be fatal[7].

Hypersensitivity pneumonitis (HP) is a complex and potentially debilitating lung disease caused by an abnormal immune response to inhaled antigens[8]. This condition is also known as extrinsic allergic alveolitis, and it is caused by a combination of genetic and environmental factors[9]. The development of HP is a multi-step process that involves the inhalation of antigens, such as dust, fumes, mould, or proteins, which trigger an immune response in susceptible individuals[10]. The

antigens are usually derived from occupational or environmental sources, such as farming, bird-keeping, or exposure to chemicals[11]. Once the antigens are inhaled, they are recognized by the immune system, which responds by activating immune cells, such as T-cells and macrophages[12]. These immune cells release inflammatory mediators, which cause inflammation and damage to the lung tissue. Symptoms of HP may include fever, shortness of breath, cough, fatigue, chest pain, wheezing and dyspnoea[13]. Untreated hypersensitivity pneumonitis can progress to serious complications such as pulmonary fibrosis and potentially life-threatening respiratory failure[14]. Prevention, early diagnosis and treatment are essential to prevent long-term lung damage and improve outcomes[8, 15].

*Cymbopogon citratus,* also known as the "lemon grass" is a unique and intriguing plant species native to tropical regions of Asia and Australia [16]. It is a perennial grass that has been widely cultivated for its culinary, medicinal, and aromatic properties. The leaves of *Cymbopogon citratus* are long and narrow, with a bright green color and a distinctive citrusy aroma [17]. Its remarkable long leaf structure has captivated botanists, naturalists, and the general public alike One of the most significant uses of lemongrass leaves is in cooking, particularly in Asian and Caribbean cuisine[18]. The leaves are often used to add flavor to soups, curries, and marinades, and are also used as a tea ingredient. The citrusy flavour of lemongrass is due to the presence of citral, a mixture of geranial and neral, which have made it a valuable ingredient in traditional medicine [19].

In addition to its culinary uses, the leaf of *Cymbopogon citratus* has also been used in traditional medicine for its anti-inflammatory and antioxidant properties. In some parts of the world, for example, tropical regions of Asia and Australia, *Cymbopogon citratus* is used in traditional medicine to treat various ailments, including cough, catarrh, fever, chest pain, and difficulty breathing[20]. *Cymbopogon citratus* contains a variety of phytochemicals, including alkaloids, flavonoids, glycosides, phenolic acids, saponins, steroids, tannins, and terpenoids[21]. These phytochemicals contribute to the plant's medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial activities[22]. *Cymbopogon citratus* was chosen as a potential therapeutic agent for dichlorvos-induced hypersensitivity pneumonitis due to its well-documented antioxidant and anti-inflammatory properties, which align with the need to counteract the oxidative stress and inflammation caused by dichlorvos toxicity. Hence, the objective of this study was to evaluate the effects of aqueous leaf extract of *Cymbopogon citratus* on dichlorvos-induced hypersensitivity pneumonitis in Wistar rats.

## MATERIALS AND METHODS

***Plant Materials*:** The *Cymbopogon citratus* leaves used in this study were collected from the University of Benin Farm Project, located in Benin City, Nigeria. The plant material was authenticated by botanists at the Department of Plant Biology and Biotechnology, Faculty of Sciences, University of Benin, Benin City, Edo State, Nigeria. A voucher specimen (voucher number: UB/MPCI/004) was lodged at the department's herbarium for future reference.

***Extract Preparation*:** *Cymbopogon citratus* leaves were oven-dried at 40$^{o}$C after air-drying for 7 days. The dried leaves were then grounded using a 2018 model mechanical grinder (Dozenmann, U.S.A). The cold maceration method was used to extract the powdered material by soaking 500g of the powdered *Cymbopogon citratus* leaf in 1 liter of water for 24 hours at room temperature [23]. The cold-macerated method was selected for extracting active compounds from *Cymbopogon citratus* leaves due to its ability to yield high-

quality extracts with minimal degradation of phytochemicals. This technique enables the extraction of a wide range of bioactive compounds, including flavonoids, saponins, and terpenoids which are known to contribute to the medicinal properties of *Cymbopogon citratus*. Additionally, cold maceration is a simple and scalable method that can be easily replicated in a laboratory setting, making it an attractive choice for our research. The soaked *Cymbopogon citratus* was filtered with the aid of cotton wool. Using evaporating dishes, the filtrate was concentrated over a hot water bath to obtain 20 g concentrated jellylike extract of *Cymbopogon citratus* leaf which was then transferred into plain specimen bottles for storage in a refrigerator at 4$^{o}$C. Acute oral toxicity of the extract was evaluated.

***Experimental animals*:** Thirty (30) adult Wistar rats of 236-256 g in weight were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria and were utilized for this experiment. The rats were housed in cages with 6 per cage, wood shavings bedding, a 12-hour light/dark cycle, 50-60% humidity, and an ambient temperature of 25-28$^{0}$C. The housing conditions were designed to minimize stress and ensure the comfort and well-being of the animals. The animals were left to acclimatize for 2 weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed manufactured by Bendel Flour Mill, Ewu, and clean water *ad libitum*. The supplier, Bendel Flour Mill, situated in Ewu, Edo State, Nigeria, delivered Standard Animal Feed in pellet form (Batch number: BMI/EWCB/002). We ensured that the nutritional value of the feed remained uncompromised by using it within the recommended time frame.

***Ethical consideration*:** Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (The approval number obtained is CMS/REC/2014/108). Each animal procedure was carried out following approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research [24].

***Induction of hypersensitivity pneumonitis***

*Hypersensitivity* pneumonitis was induced in the test animals by exposure to dichlorvos at 100 mg/m$^3$, delivered as 2 puffs (10 ml/puff) via a handheld sprayer in a fume distributor glass-chamber (FDGC), with daily 1-hour exposure for 30 days[25]. The dichlorvos were manufactured by DZT Chemicals, Sango-Ota, Ogun State, Nigeria (Product Code: DVN-026, Batch number: DCN/8908/006CN/007). We ensured that the concentration and dose of dichlorvos used in this study were accurate and reproducible, and we provided these details to facilitate replication by other researchers. A pilot study was done on the 28$^{th}$ day of the experiment, utilizing a sample of 5 animals (n=1 per group), which confirmed the presence of hypersensitivity pneumonitis.

***Experimental design*:** In this study, 30 animals were divided into 5 groups comprising 6 rats per group. Animals in group A which served as control received standard feed and clean water *ad libitum*. Animals in group B were exposed to dichlorvos only via inhalation at 100 mg/m$^3$, delivered as 2 puffs (10 ml/puff) via a handheld sprayer in a fume distributor glass chamber (FDGC). Animals in group C received 500 mg/kg body weight per day (BWT/D) of *Cymbopogon* citratus. Animals in group D were exposed to dichlorvos via inhalation and received 250 mg/kg BWT/D (low dose) of *Cymbopogon* citrates. Animals in group E were exposed to dichlorvos via inhalation and received 500 mg/kg BWT/D (high dose) of *Cymbopogon citratus***.** We selected the dosages of *Cymbopogon* citrates for groups D and E based on previous studies that have demonstrated the safety and efficacy of this plant extract in animal models. Our selection of these dosages was also guided by the results of our pilot study. The dosages were given for 30 consecutive days via an orogastric tube.

***Method of sacrifice and sample collection:*** On the 30$^{th}$ day of exposure to dichlorvos, the animals were euthanized under chloroform anesthesia and the lung of each rat was excised and immediately fixed in 10% formal saline for 24 hours to prevent tissue degradation and autolysis before the histological procedures. The tissues were sectioned into about 5 µm thick sections and processed according to the method of Drury and Wallington [26]. The thin tissue sections 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 for 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 a magnification of x40.

**RESULTS**:

Histological findings in all the experimental groups are presented in Figure 1. It was observed that the photomicrograph of the control group (group A) showed normal features of the lungs, including alveoli, interstitial space, bronchial artery, bronchiolo-alveolar lymphoid tissue, and respiratory bronchioles.

It was found that in the rats exposed to 2 puffs of dichlorvos (group B), there were severe alveolar hemorrhage, bronchiolar hemorrhage and heavy interstitial infiltrates of inflammatory cells. We found that the group given 500 mg extract of *Cymbopogon citratus* only (group C) presented the normal architecture of alveoli, terminal bronchiole, and activated mononuclear phagocyte tissue. Also, it was observed that in the animals given 250 mg/kg body weight of extract of *Cymbopogon citratus* and exposed to 2 puffs of dichlorvos (group D), there were normal architecture of alveoli, bronchial artery, bronchial vein, terminal bronchiole and activated cells of the mononuclear phagocyte system. Finally, we noted that the group given 500 mg/kg body weight of extract of *Cymbopogon citratus* and exposed to 2 puffs of dichlorvos (group E) presented normal architecture of alveolar, respiratory bronchiole and interstitial infiltrates of inflammatory cells.

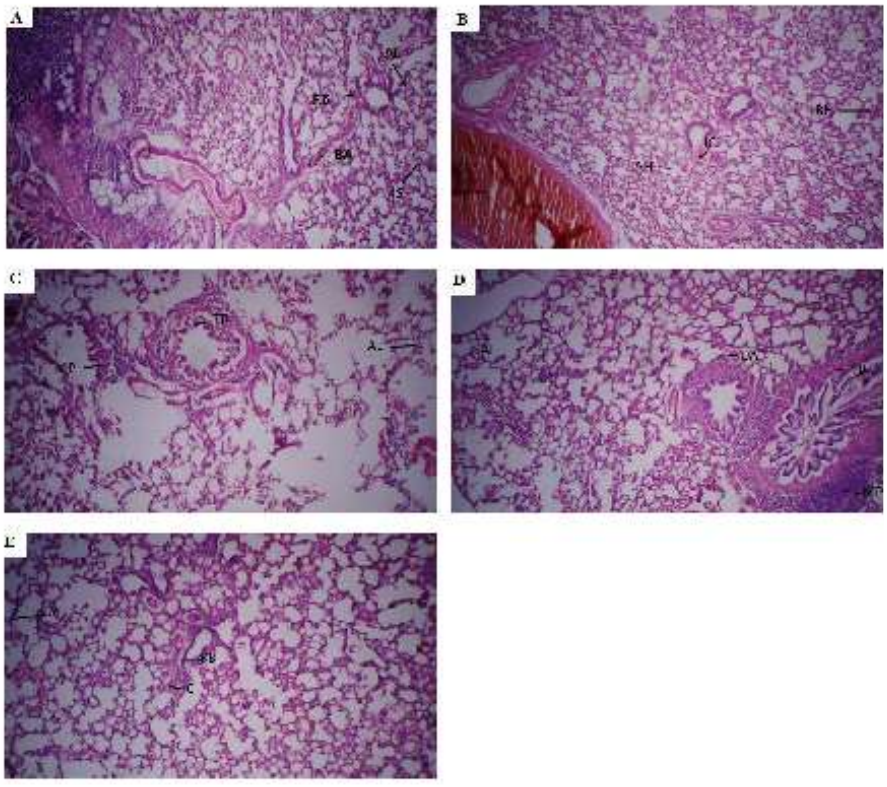


**Figure 1:** Representative photomicrographs of H&E sections of the lungs of the experimental rats: 'A' (Control group) showed normal bronchiolo-alveolar lymphoid tissue 'BL', alveoli 'AL', normal bronchial artery 'BA', respiratory bronchiole 'RB' and interstitial space 'IS'. 'B' showed rats exposed to 2 puffs of dichlorvos (10 ml/puff) only via inhalation daily for 30 days showing bronchiolar hemorrhage 'BH', alveolar hemorrhage 'AH', and interstitial inflammatory cells infiltrates (IC): 'C'

showed rats administered 500 mg/kg body weight per day of *Cymbopogon citratus* aqueous leaf extract only daily for 30 days showing normal architecture of mononuclear phargocyte tissue 'MP', alveoli 'AL' and normal architecture of terminal bronchiole 'TB'. 'D' showed rats exposed to 2 puffs of dichlorvos fumes via inhalation and received 250 mg/kg body weight of *Cymbopogon citratus* aqueous leaf extract daily for 30 days showing normal architecture of alveoli 'AL', bronchial artery 'BA', activated cells of the mononuclear phagocyte system 'MP' and terminal bronchiole 'TB'. 'E' showed Group E exposed to 2 puffs of dichlorvos fumes via inhalation and received 500 mg/kg body weight of *Cymbopogon citratus* aqueous leaf extract daily for 30 days showing normal architecture of alveoli 'AL', normal respiratory bronchiole 'RB', and interstitial infiltrates of inflammatory cells 'IC'.

## DISCUSSION

*Cymbopogon citratus* has been reported to possess various medicinal uses, including anti-tussive, anti-inflammatory, and anti-histamine properties[27]. Against this background, this study was conducted to evaluate the effects of aqueous leaf extract of *Cymbopogon citratus* on dichlorvos-induced hypersensitivity pneumonitis in adult Wistar rats.

Observations based on photomicrography show that dichlorvos caused bronchiolar haemorrhage, alveolar haemorrhage, and interstitial infiltrates of inflammatory cells (evidence of hypersensitivity pneumonitis) in the exposed animals. There was florid activation of the lung tissue of the rats that were exposed to dichlorvos alone which occurred as a result of the body sensing extraneous substances (excess accumulation of dichlorvos fumes) leading to the activation of lymphoid tissues to get rid of it. *Cymbopogon citratus* had no negative effects on the histology of the lungs as the activated mononuclear phagocyte tissue, alveoli, and terminal bronchioles were found to be histologically normal in the rats that were administered only the extract. Low doses of *Cymbopogon citratus* caused normal architecture of the alveoli, bronchial artery, terminal bronchiole and activated cells of the mononuclear phagocyte system. Furthermore, high doses of the extract revealed preservation of normal alveoli and respiratory bronchiole architecture, indicating protective effects against dichlorvos-induced hypersensitivity pneumonitis. Hypersensitivity pneumonitis was completely prevented and the accumulated dichlorvos particulate matters were cleared. The phytochemicals present in *Cymbopogon citratus*, particularly flavonoids, saponins, and alkaloids, likely contributed to its protective effects against dichlorvos-induced hypersensitivity pneumonitis. Flavonoids, for instance, may have reduced inflammation by inhibiting pro-inflammatory cytokines e.g., tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-12 (IL-12) and scavenging free radicals[28], while saponins may have modulated immune responses and reduced oxidative stress[29].

The key phytochemicals in *Cymbopogon citratus*, including flavonoids and saponins, have been shown to exhibit anti-inflammatory and antioxidant properties, which may have contributed to the observed protective effects. Our findings are consistent with previous studies that have demonstrated the protective effects of *Cymbopogon citrates* against toxicant-induced lung damage. For example, a study by Weli *et al.*,[30] found that *Cymbopogon citratus* extract reduced lung inflammation and oxidative stress in mice exposed to cigarette smoke.

The phytochemicals in *Cymbopogon citratus* may interact with specific cellular pathways or molecular targets to reduce lung inflammation, including the Nuclear Factor Kappa B (NF-KB) and Nuclear Factor Erythroid 2-related Factor 2 (Nrf2 pathways, which regulate inflammatory responses and antioxidative defences. Further studies are needed to elucidate the precise mechanisms involved.

The different doses of *Cymbopogon citratus* extract used in this study may have influenced the effects on the outcome measured by modulating the bioavailability and pharmacokinetics of the phytochemicals. Lower doses may have resulted in greater bioavailability and enhanced protective effects. Our findings suggest that *Cymbopogon citratus* extracts may have potential therapeutic

applications in treating or preventing lung diseases caused by dichlorvos exposure.

## CONCLUSION

*Cymbopogon citratus* has ameliorative effects against dichlorvos-induced bronchiolar haemorrhage, alveolar haemorrhage, and activation of lymphoid tissue. *Cymbopogon citratus* is therefore valuable in combating hypersensitivity pneumonitis.

**Conflict of interest:**

The authors, Ehi-Omosun, Mabel Bilu and Olise, Augustina Nkechi hereby declare no potential conflicts of interest in this manuscript.

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