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CAN *CYPERUS ESCULENTUS* AMELIORATE LUNG DAMAGE? PRELIMINARY HISTOLOGICAL ASSESSMENT IN ARSENIC TRIOXIDE INDUCED WISTAR RATS

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ABSTRACT

Inhalation of metal particles or fumes can be toxic and cause various health problems. Prolonged exposure to metal inhalation can lead to many respiratory issues. This study investigated the protective effects of ethanol tuber extract of Cyperus esculentus (ETECE) against arsenic trioxide (ATO)-induced lung damage in Wistar rats. A total of 49 Wistar rats (n=7), weighing 190-210g, were randomly assigned to seven groups (all groups received feed and administration): Group A (control): received 1ml of distilled water, Group B: received 10 mg/kg body weight of ATO only, Group C: received 200 mg/kg body weight of ETECE + ATO, Group D: received 400 mg/kg body weight of ETECE + ATO, Groups E and F received 200 and 400 mg/kg body weight of ETECE respectively, and Group G: received ATO + 100 mg/kg body weight of Vitamin C. Administration lasted for 60 days, after which the animals were humanely sacrificed. The lungs were meticulously harvested, fixed in 10% neutral buffered formalin and then processed for histological examination using haematoxylin and eosin (H&E) staining. Histopathological examination revealed that exposure to ATO induced significant pulmonary damage, characterized by interstitial infiltrates of inflammatory cells, congestion, severe bronchiolar ulceration, and vascular ulceration. Conversely, treatment with escalating doses of ETECE remarkably attenuated these detrimental effects. Notably, ETECE demonstrated a comparable protective effect to that of Vitamin C, a well-established antioxidant. In summary, the findings of this study suggest that ETECE exhibits mitigating effects against ATO-induced lung damage in Wistar rats, thereby underscoring its potential as a pulmonoprotective substance.

Keyword: *C. esculentus*; arsenic trioxide; inflammatory cells; vascular ulceration.

INTRODUCTION

The lungs, being the vital organs responsible for respiration, are susceptible to damage caused by pulmonotoxic chemicals, which can lead to pulmonary toxicity. This toxicity can manifest as inflammation, scarring, or other complications (Courcot et al., 2021). The lungs are constantly exposed to toxic metals originating from various sources, including the environment, food, consumer products, and pharmaceuticals. Specifically, the bronchial and alveolar epithelial cells in the lungs are primary targets and, therefore, are particularly vulnerable to the toxic effects induced by these chemicals (Aspal and Zemans, 2015).

Arsenic, notorious for its toxic properties, has been recognized as a potent poison throughout history. However, the escalating levels of arsenic pollution in the atmosphere have transformed this issue into a pressing public health concern (Zhang et al., 2020). The primary anthropogenic sources contributing to arsenic pollution are industrial activities, the application of arsenic-based pesticides, and the combustion of coal, which collectively exacerbate environmental contamination and pose significant health risks (Wai et al., 2016). During high-temperature combustion processes, arsenic is predominantly released as arsenic trioxide (ATO) (Gong et al., 2019). Notably, approximately one-third of the arsenic emitted from coal combustion vaporizes directly into the atmosphere, while the remaining arsenic is enriched in fine particles that can be transported over extensive distances, resulting in widespread environmental pollution (Tanda et al., 2019). Coal combustion, particularly during the cold season, frequently triggers severe hazy days, leading to prolonged arsenic emissions into the atmosphere (Liu et al., 2020). These severe haze events often persist for 3-5 days, resulting in extreme air pollution (Zheng et al., 2016). Moreover, acute exposure to arsenic has been linked to the development of non-malignant respiratory diseases, including conditions such as bronchitis and asthma (Powers et al., 2019; Sanchez et al., 2016; Shih et al., 2019). Epidemiological studies have further established a link between arsenic exposure and increased mortality due to lung disease, as well as the exacerbation of respiratory symptoms and decreased lung function (Rahman et al., 2022; Siddique et al., 2020).

Cyperus esculentus, widely known as tigernut, earth almond, or yellow nutsedge, is a cultivated crop within the Cyperaceae family (Sanchez-Zapata et al., 2021). As a nutrient-rich tuber, it serves as an excellent energy source, boasting significant amounts of starch, fat, protein, sugar, and essential dietary minerals, thereby making it a valuable and nutritious food resource (Raphael et al., 2010). *C. esculentus* is a widely recognized plant in Nigeria, with diverse local names across ethnic groups, including "Aya" in Hausa, "Imumu" in Yoruba, and "Ofio" in Igbo (Omode et al., 1995). This versatile plant is utilized in various forms by Nigerians, who consume it fresh, dried, roasted, or as a key ingredient in the traditional beverage "Kunu" (Oladele and Aina, 2007). In Nigeria, the plant is mainly cultivated in the middle belt and northern regions, where three distinct varieties - black, brown, and yellow - are grown. While all three varieties are cultivated, the yellow and brown varieties are more commonly available in markets. *C. esculentus* boasts an impressive nutritional profile, rich in antioxidants such as vitamin E, vitamin C, and quercetin, as well as essential minerals including zinc, potassium, and phosphorus (Allouh et al., 2015). In addition to its nutritional value, *C. esculentus* has been traditionally revered for its potential to enhance male fertility and sexual wellness. Research has demonstrated that it can augment libido, improve sexual performance, and even restore sexual function in individuals with pre-existing sexual abnormalities, thereby positioning it as a valuable natural remedy for promoting reproductive health (Saheed et al., 2016).

Beyond its numerous health benefits, *C. esculentus* has also been traditionally utilized in treating urinary tract and bacterial infections, as well as reducing the risk of colon cancer when consumed (Adejuyitan et al., 2009). In recent years, researchers have shown increasing interest in exploring the potential of medicinal plants with antioxidant properties, such as *C. esculentus*, to counteract metal toxicity (Sudjarwo et al., 2017). This present study aimed to investigate the protective effects of ethanol tuber extract of *C. esculentus* (ETECE) against ATO-induced lung damage in Wistar rats.

MATERIALS AND METHOD

Collection, Verification, and Processing of Botanical Samples:

C. esculentus tubers were sourced from the New Benin Market in Benin-City, Edo State,

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Nigeria. For authentication purposes, a sample was submitted to the University of Benin's Department of Plant Biology and Biotechnology, where it was positively identified and assigned the herbarium number UBH-C419. After verification, the tubers underwent thorough washing with tap water, air-drying to remove excess moisture, and pulverization into a fine powder. A 150g portion of the powdered tubers was then soaked in 1000ml of 50% ethanol for 72 hours. The crude ethanol extract was filtered using a Buchner funnel and Whatman No.1 filter paper to obtain a clear filtrate, which was subsequently freeze-dried using the method described by Kumar (2019) at the University of Benin's Natural Product Research Laboratory. The freeze-dried extract was stored in a refrigerator at -4°C pending further analysis.

Animal Models and Treatment Administration:

A total of 49 adult Wistar rats, weighing between 190 and 210g, were utilized for this study. These rats were bred in the anatomy animal house at the School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The animals were provided with unrestricted access to food and water and were housed in a controlled laboratory environment designed to ensure their optimal comfort and well-being. The laboratory conditions were carefully maintained within a narrow range, with a temperature of 28 ± 2°C, relative humidity of 50 ± 5%, and a 12-hour light-dark cycle. The rats were randomly assigned to seven groups, each consisting of seven rats. Following a 2-week acclimatization period, the animals received predetermined dosages of ETECE and ATO via oral gavage, using a modified version of the binge-drinking model developed by Carson and Pruett (1996). The dosages were determined based on the LD_{50} values obtained using Lorke's method (1983). The treatment groups (received feed in addition to administration) and were as follows: Group A (control): 1ml of distilled water, Group B: 10 mg/kg body weight of ATO only, Group C: 200 mg/kg body weight of ETECE and ATO, Group D: 400 mg/kg body weight of ETECE and ATO, Group E: 200 mg/kg body weight of ETECE only, Group F: 400 mg/kg body weight of ETECE only and Group G: ATO and 100 mg/kg body weight of Vitamin C.

Animal Euthanasia and Tissue Harvesting:

After completing the 60-day treatment regimen, the rats were humanely euthanized using ketamine anesthesia. Anesthesia was induced by placing cotton wool soaked in approximately 30ml of ketamine in an enclosed container for 2-5 seconds. Once anesthetized, each rat was positioned on the dissection table, and a thoraco-abdominal incision was made to access the thoracic viscera. The lung was then carefully excised and immediately preserved in 10% formalin solution within a universal container, in preparation for subsequent histopathological analysis.

*Histological Examination and Tissue Pathology Analysis***:**

Following fixation, the lung tissue underwent routine histological processing. This process entailed dehydration in a graded ethanol series (70-100%), followed by clearing with xylene and embedding in paraffin wax. Thin sections were then cut from the embedded tissue, stained with hematoxylin and eosin (H&E) according to the protocol described by Drury and Wallington (1980), and examined under a light microscope to evaluate any histological alterations.

RESULTS

The lung of control group (Group A) showed normal alveolar, interstitial space, bronchiole and bronchial artery (Figure 1). The lung of Group B (ATO only) showed interstitial infiltrates of inflammatory cells, congestion, severe bronchiolar ulceration and vascular ulceration (Figure 2). The liver of Group C (200 mg/kg of ETECE and ATO) showed normal alveoli, terminal bronchiole and active interstitial congestion (Figure 3). The liver of

Group D (400 mg/kg of ETECE and ATO) showed normal alveoli, active interstitial congestion and bronchiolar dilation (Figure 4). The lung of Group E (200 mg/kg of ETECE only) showed normal alveola, activated cells of the mononuclear phagocyte system and terminal bronchiole (Figure 5). The lung of Group F (400 mg/kg of ETECE only) showed normal alveoli, active interstitial congestion and activated mononuclear phagocyte system (Figure 6). The lung of Group G (Vitamin C and ATO only) showed normal alveoli, bronchial artery and bronchiolar dilatation (Figure 7).

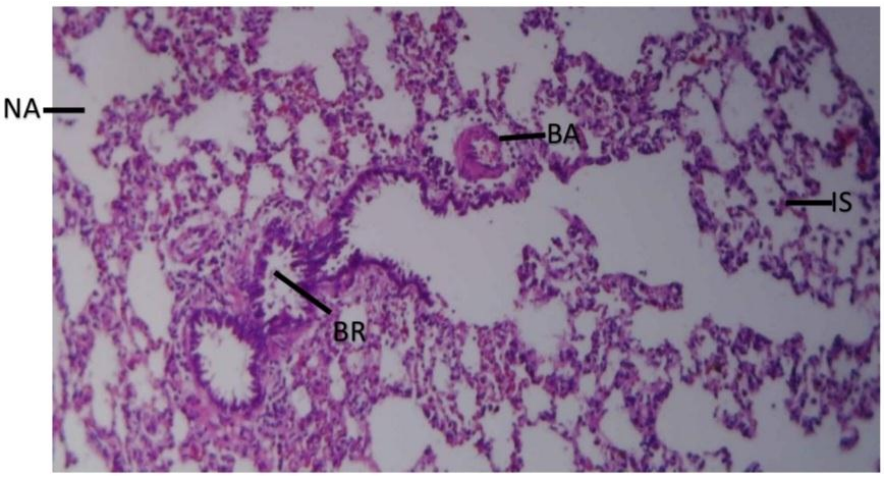

Figure 1. Photomicrograph of the lung of the control group (group A) showing normal alveolar (NA), interstitial space (IS), bronchiole (BR) and bronchial artery (BA). H and E 100x.

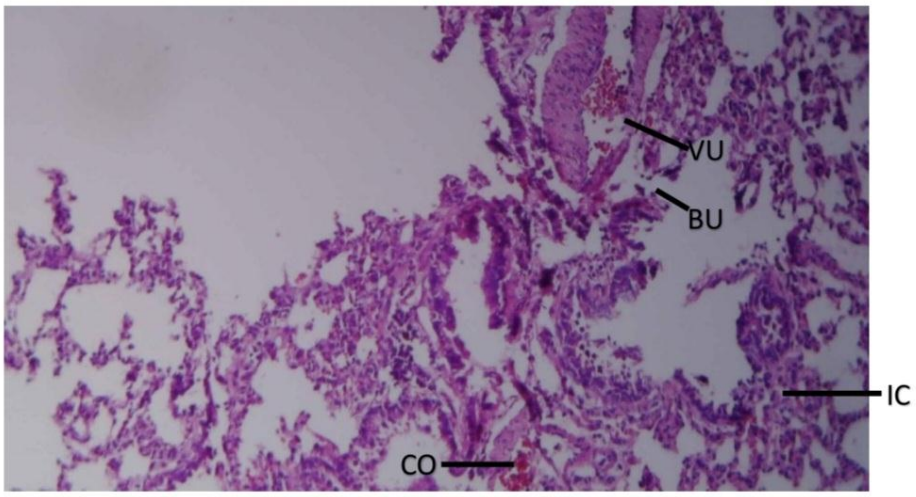

Figure 2. Photomicrograph of the lung of Group B (ATO only) showing interstitial infiltrates of inflammatory cells (IC), congestion (CO), severe bronchiolar ulceration (BU) and vascular ulceration (VU). H and E 100x.

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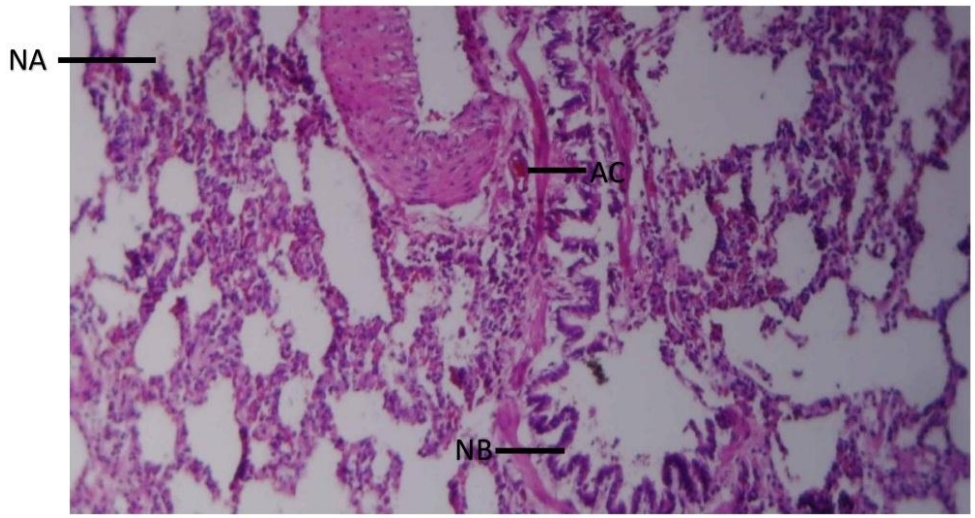

Figure 3. Photomicrograph of the lung of Group C (200 mg/kg of ETECE and ATO) showing normal alveoli (NA), terminal bronchiole (NB) and active interstitial congestion (AC). H and E 100x.

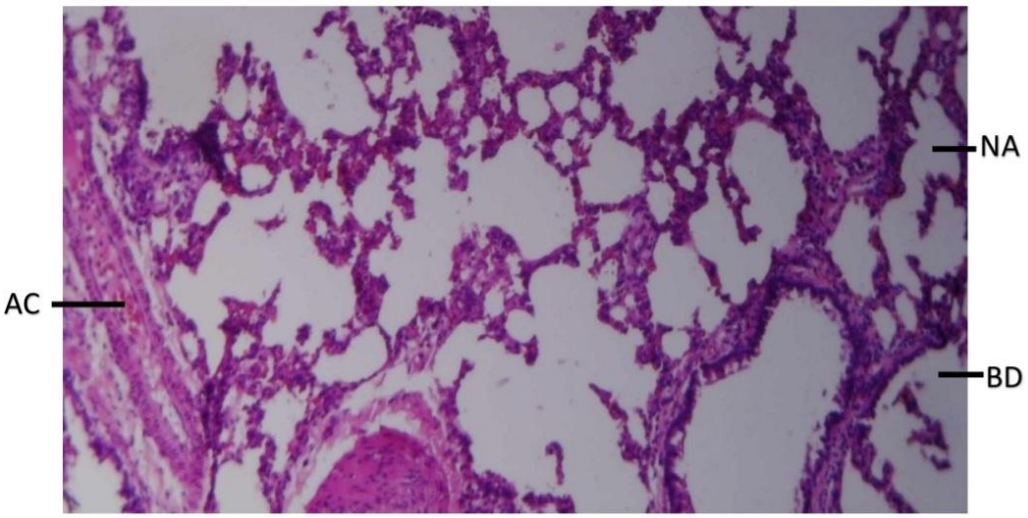

Figure 4. Photomicrograph of the lung of Group D (400 mg/kg of ETECE and ATO) showing normal alveoli (NA), active interstitial congestion (AC) and terminal bronchiolar dilation (BD). H and E 100x.

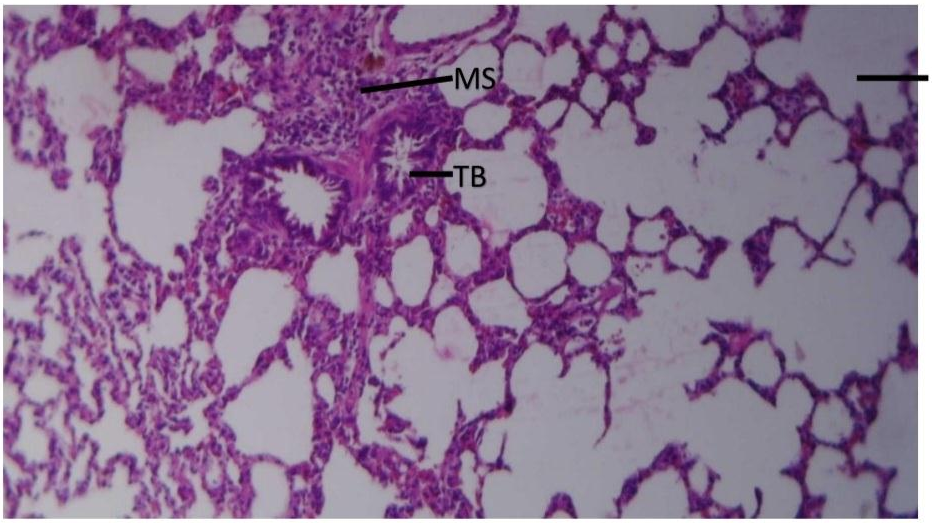

Figure 5. Photomicrograph of the lung of Group E (200 mg/kg of ETECE only) showing normal alveoli (NA), activated cells of the mononuclear phagocyte (MS) and terminal bronchiole (TB). H and E 100x.

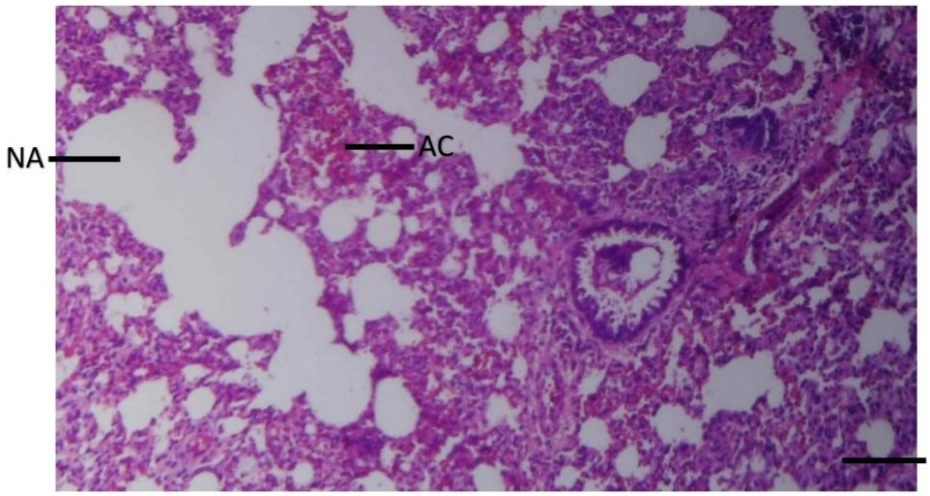

Figure 6. Photomicrograph of the lung of Group F (400 mg/kg of ETECE only) showing normal alveoli (NA), active interstitial congestion (AC) and activated mononuclear phagocyte system (MS). H and E 100x.

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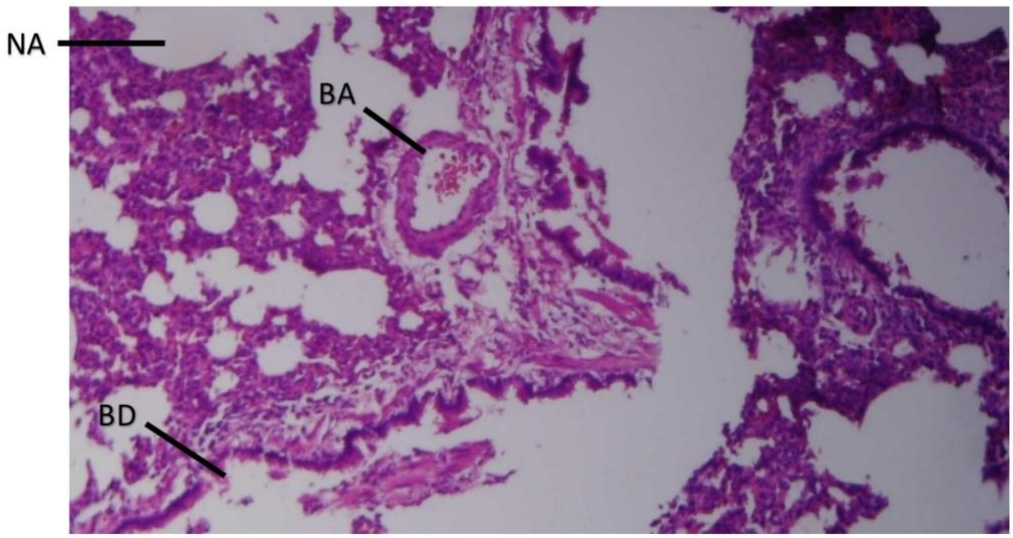

Figure 7. Photomicrograph of the lung of Group G (Vitamin C and ATO) showing normal alveoli (NA), bronchial artery (BA) and bronchiolar dilation (BD). H and E 100x.

DISCUSSION

Inhalation of arsenic from the atmosphere allows it to enter the human respiratory system, potentially passing through the trachea and bronchi and accumulating in the lung alveoli (Xie et al., 2021). As a result, the health risks associated with arsenic inhalation are a significant concern, particularly for individuals with compromised immune systems or pre-existing vulnerabilities. The alveolar epithelium of the lungs serves as a delicate interface between the internal and external environments. This thin, yet vital, barrier facilitates gas exchange while being continually exposed to potentially hazardous environmental stimuli (Knudsen and Ochs, 2018). The alveolar epithelium is comprised of two primary cell types: type 1 (AT-I) and type 2 (AT-II) alveolar cells, with AT-I cells occupying approximately 95% of the lung's surface area. Notably, the alveolar barrier consists of both endothelial and epithelial cells; however, research suggests that alterations in epithelial cell permeability alone can lead to pulmonary edema, underscoring the critical role of epithelial cells in maintaining lung function (Frank, 2012). ATO, a potent toxicant, causes pulmonotoxicity through various mechanisms, such as oxidative stress, inflammation, apoptosis and disruption of cellular signaling pathways (Shi et al., 2019).

C. *esculentus* is a plant celebrated for its exceptional antioxidant properties, earning its reputation as a valuable medicinal herb. As a natural antioxidant, C. *esculentus* possesses the ability to scavenge free radicals and protect body organs from oxidative stress, thereby mitigating potential damage (Belewu, 1996). *C. esculentus* exhibits a diverse range of therapeutic and biological effects, including antioxidant, anti-inflammatory, aphrodisiac, and anti-diabetic properties, among others. These multifaceted effects collectively contribute to enhanced overall well-being and improved health outcomes, underscoring the potential of *C. esculentus* as a valuable adjunct in promoting human health (Edo et al., 2023).

In this study, histological examination of lung sections from control rats, which received standard feed and water, revealed normal lung architecture. Similarly, lung sections from rats treated with graded doses (200 and 400 mg/kg body weight) of ETECE (groups E and F) also showed normal histological architecture. Notably, activation of the mononuclear

phagocyte system was observed in these treated groups, suggesting a boost in the local immune response within the lungs. This is in line with study by Oladele et al. (2017), which showed that extract of *C. esculentus* activates immune cells, such as natural killer cells and T-cells, which play a crucial role in the immune response and by Ade-Omowaye et al, (2013), who reported that the extract increases the activity of macrophages in Wistar rats, which are essential for the elimination of pathogens. Histological examination of lung sections from rats treated with ATO (group B) alone revealed significant pathological changes, including interstitial infiltrates of inflammatory cells, congestion, severe bronchiolar ulceration, and vascular ulceration. These findings are consistent with previous studies, such as Wang (2021), which demonstrated that ATO exposure in Wistar rats led to lung damage and inflammation, characterized by structural changes in lung tissue, including damage to alveolar epithelial cells and capillary endothelial cells. Furthermore, Kitchin and Ahmad (2003) reported similar findings, including significant infiltration of inflammatory cells, such as neutrophils and macrophages, which contribute to tissue damage and oxidative stress in the lungs of Wistar rats exposed to ATO. Histological examination of lung sections from rats treated with 200 mg/kg body weight of ETECE plus ATO (group C) revealed normal lung architecture, characterized by well-defined alveolar sacs, intact interstitial spaces, and normal blood vessels and bronchioles. Similarly, lung sections from rats treated with 400 mg/kg body weight of ETECE plus ATO (group D) and those treated with Vitamin C plus ATO (group G) showed normal tissue architecture, with the additional observation of mild bronchiolar dilation. This finding suggests that both ETECE and Vitamin C, a well-known antioxidant, possess protective properties that can mitigate the harmful effects of ATO exposure on the lungs, particularly by reducing oxidative stress and inflammation (Oladele et al., 2017). The antioxidant and anti-inflammatory properties of ETECE may contribute to its potential therapeutic benefits in alleviating ATO-induced lung damage.

CONCLUSION

Arsenic trioxide (ATO) exposure induced significant histological lung damage, but concurrent treatment with graded doses of tigernut extract (ETECE) demonstrated a comparable, if not superior, protective effect to that of Vitamin C, a well-established antioxidant. Notably, ETECE treatment achieved a remarkable amelioration of ATO-induced lung damage, suggesting its potential as a pulmonoprotective agent. These findings provide compelling evidence that ETECE possesses pulmonoprotective properties, warranting further investigation into its therapeutic potential as a protective agent against lung damage.

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