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Ellagic acid alleviates pulmonary inflammation and oxidative stress in mouse model of diisononyl phthalate-induced asthma

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ABSTRACT

Polyvinyl chloride is plasticized using diisononyl phthalate (DiNP), and exposure to phthalates has been linked to the emergence of asthma and allergies. The adjuvant impact of DiNP exposure results in allergic airway inflammation. In the current study, we looked into how ellagic acid (ELA) impacted asthma brought on by DiNP. Male BALB/c mice (n=40, 20-30 g) were divided into 4 groups of 10 mice each, and the following treatments were given to each group: group 1 (control) received saline orally for 30 days; group 2 (ELA) received 10 mg/kg of ELA (oral) for 30 days; group 3 (DiNP & ELA) received 10 mg/kg of ELA (oral) for 7 days prior to DiNP (50mg/kg) exposure (intraperitoneal and intranasal); group 4 (DiNP) received 50 mg/kg DiNP. After the last administration, mice were sacrificed, lungs were removed and their bronchoalveolar lavage fluid was collected which was used for biochemical and histopathological analysis. The mice given DiNP had changes in their histoarchitecture, inflammatory cells, antioxidant status, and inflammation markers. Malondialdehyde (MDA) and inflammatory biomarkers (NO, MPO) were considerably higher ($p< 0.05$) in the lungs of DiNP-treated mice than in the control group. In the lungs, DiNP reduced the concentration of non-enzymatic antioxidants (GSH and AA), and the activity of enzymatic antioxidants (SOD, CAT, and GST). DiNP also altered inflammatory cells (eosinophils, neutrophils, lymphocytes, monocytes, leucocytes) in the BALF. ELA administration ameliorated these changes. Histopathological analysis revealed airway inflammation characterized by inflammatory cell infiltration, oedema, hemorrhage, and constricted alveoli space. Additionally, the mice co-treated with ELA and DiNP experienced mild inflammation of the alveoli and interstitial spaces as well as mild thickening of the alveolar septae. ELA offered a protective effect against DiNP-induced allergic asthma in mice.

Keywords: *Phthalates; diisononyl phthalates; asthma; lungs; ellagic acid and mice*

INTRODUCTION

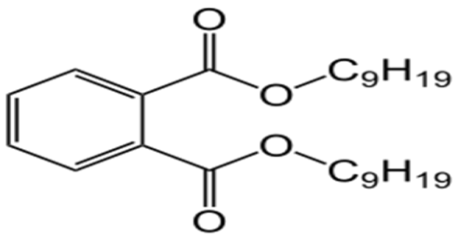
Asthma is a common allergy condition that is becoming more common worldwide affecting 300 million people. Around the world, asthma mortality and morbidity are more prevalent in high-income countries than in low- and middle-income ones, where diagnosis and treatment are more difficult. In children and young people, it is becoming more complex and severe (Wu et al., 2019). Airway remodelling, mucus oversecretion, and inflammatory airways are all symptoms of asthma. The quantity of eosinophils and lymphocytes increases, along with their

infiltration of the mucosa and submucosa, which causes airway hyperresponsiveness (AHR). For these reasons, coughing, wheezing, shortness of breath, and tightness in the chest are all clinical symptoms of asthma. Persistent exposure to allergens including viruses, cigarette smoke, air pollution, stress, and environmental pollutants increases the risk of developing asthma (Pawankar, 2014). Reactive oxygen or nitrogen species (RONS) build up in the pulmonary system can cause signalling pathways implicated in the pathogenesis of asthma to be activated (Jesenak et al., 2017). Additionally, it's typical to link asthmatic patients' functional and structural abnormalities to an elevated degree of oxidative stress (Lewis et al., 2021). Despite the fact that allergen exposure is a risk factor for developing asthma, the importance of irritant or chemical-induced asthma cannot be overstated. Irritants damage the airway epithelium, activate various regulatory molecules, and activate cytokines, which can also drive effector cells to migrate into the lungs and produce cytokines and mediators. Given that many asthma patients experience both allergic and non-allergic reactions, there may be cross-talk between allergic and non-allergic molecular pathways (Vincent et al., 2017).

Personal care goods, food packaging, children's toys, medications, nutritional supplements, cleaning supplies, lubricants, pesticides, solvents, adhesives, and paints all contain phthalates, which have been determined to be environmental pollutants (Chen et al., 2012). Polyvinyl chloride (PVC) is plasticized using a number of phthalates, including diisononyl phthalate (DiNP) as shown in Figure 1, diisodecyl phthalate (DiDP), and di-2-ethylhexyl phthalate (DEHP) (Jaakkola & Knight, 2008). Animals exposed to these phthalates are more likely to develop asthma and allergies (Bornehag and Nanberg, 2010). Through Th2 polarisation, endocrine disrupting substances (EDS) such as tributyltin chloride (TBT) and benzophenone (BP) worsen airway inflammation (Kato et al., 2006). Inflammation of the airways may be caused directly by phthalates like endocrine disrupting chemicals (EDC) (Dodson et al., 2012). In particular, DiNP exposure causes allergic airway inflammation and has an adjuvant effect in young rats (Chen et al., 2015). (Larsen et al., 2002).

Figure 1

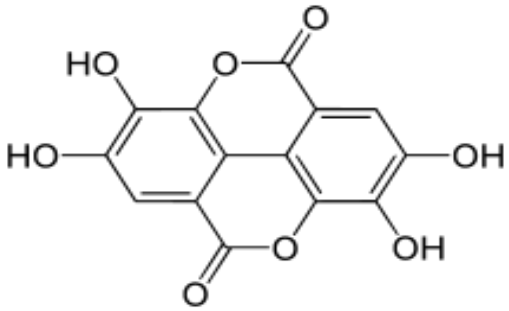
Chemical Structure of Diisononyl phthalate (Bis(7-methyloctyl) benzene-1,2-dicarboxylate)



Note. Adapted from "Subacute exposure to di-isononyl phthalate alters the morphology, endocrine function, and immune system in the colon of adult female mice," by K. Chiu, S.T. Bashir, R.A. Nowak, W. Mei & A.J. Flaws, 2020, *Scientific Report*, *10,* 18788. https://doi.org/10.1038/s41598-020-75882-0

Figure 2

Chemical Structure of Ellagic acid (2,3,7,8-Tetrahydroxy[1]benzopyrano[5,4,3-cde][1]benzopyran-5,10-dione)



Note. Adapted from "Ameliorative effects of ellagic acid on maximal electroshock and pentylenetetrazole-induced seizures in mice," by A. Khazaei, S.M.T. Mansouri, A. Siahpoosh, B. Ghorbanzadeh, S. Salehi & M.J. Khodayar, 2019, *Jundishapur Journal of Natural Pharmaceutical Products*, *14*(3). https://doi.org/10.5812/jjnpp.80039

In the treatment of asthma, the use of antioxidants or substances that trigger the endogenous antioxidant response has been very successful (Mishra et al., 2018). According to several studies, eating a diet high in antioxidants, such as fruits and vegetables, and taking vitamins A, C, and E helped to manage asthma symptoms (Patel et al., 2006; Hosseini et al., 2017). Additionally, natural phytocompounds with antioxidant activity are thought to be a viable asthma treatment. In pre-clinical trials, 6-gingerol from ginger rhizomes was discovered to lessen asthma in mice using a house dust mite model by lowering oxidative stress, reducing inflammation and inflammatory cells, and reorienting the histoarchitecture of the lungs (Ajayi et al., 2022).

Ellagic acid (ELA) as shown in Figure 2, is a naturally occurring polyphenolic compound that is found as ellagitannins, which are hydrolysable tannins, in various fruits, nut galls, and plant extracts. Raspberries, strawberries, grapes, pomegranate, black currants, camu-camu, mango, guava, walnuts, almonds, longan seeds, and green tea are some examples of these foods (Soong et al., 2006 According to studies (Choi et al., 2009; Promsong et al., 2014; Saba et al., 2013), ELA possesses antiallergic, anti-inflammatory, and pneumoprotective properties. Due to its reported biological actions, we hypothesised that ellagic acid (ELA) could reduce oxidative stress, inflammation, and lung histoarchitecture, hence alleviating DiNP-induced asthma. We confirmed the effects

of ELA on asthma caused by DiNP in the current investigation.

METHODOLOGY

Chemicals and reagents

ELA and DiNP was supplied by AK Scientific, USA. Thiobarbituric acid (TBA), hydrogen peroxide, trichloroacetic acid (TCA), Folinn-Ciocalteu reagent, O-dianisidine and all other chemicals used for the other assays were of pure grade and were gotten from Sigma-Aldrich, Missouri, USA.

Experimental animals

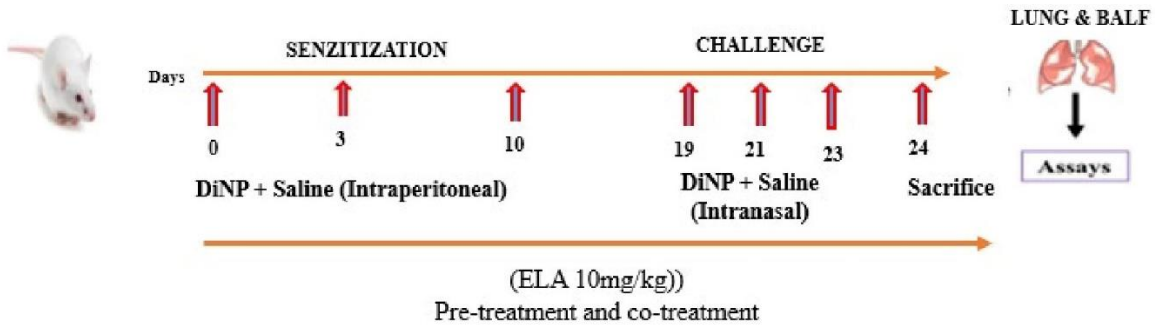
For this study, forty (40) healthy male BALB/c mice weighing 20–30 g was employed. The animals were from the University of Ibadan's Veterinary Anatomy Department's animal holdings. They were raised at Ajayi Crowther University Oyo's Department of Biochemistry. Prior to the start of the study, the mice were given a week of acclimatization and were given unlimited access to standard laboratory food and water *ad libitum*. Mice were kept in a natural environment with a 12-hour cycle of light and darkness at a temperature of 22–25 °C. The Ethical Review Committee of the Faculty of Natural Sciences at Ajayi Crowther University approved the study's experimental design and assigned it the ethical number FNS/ERC/23/001E. The National Academy of Science's (NAS) "Guide for the Care and Use of Laboratory Animals" (as reported by the National Institute of Health) was followed in providing the mice with humane treatment.

Experimental design

The mice were divided into four (4) groups of ten (10) each, and each group received the following treatment: group 1 (control) mice received saline orally for 30 days; group 2 (ELA) mice received 10 mg/kg of ELA (oral) for 30 days; group 3 (DiNP and ELA) mice received 10 mg/kg of ELA (oral) for seven (7) days prior to exposure to 50 mg/kg DiNP (intraperitoneal and intranasal (IP and IN). On the basis of earlier investigations, the dose for ELA and DiNP was chosen (Favarin et al., 2013; Hwang et a., 2017). The mice were sacrificed 24 h after the last administration which was on day 24 after DiNP administration was terminated on day 23. The schematic diagram for experimental design is as shown in Figure 3.

Figure 3

DiNP-induced Asthma Experimental Design



Induction of asthma

The intraperitoneal injection of 50 mg/kg of DiNP in 0.2 mL of saline on day 0 was the main method used to sensitize the mice. On days 3 and 10, intraperitoneal injections of 50 mg/kg of DiNP dissolved in saline were used to secondarily sensitize the mice. On days 19, 21, and 23, the mice were given an intranasal injection of 50 mg/kg of DiNP diluted in 50 mL of saline. The intranasal injection of 50mg/kg of DiNP was 50µL.

Collection of bronchoalveolar lavage fluid (BALF)

The procedure outlined by Ajayi et al. (2022) was used to collect the bronchoalveolar lavage fluid. The mice were given ketamine anaesthesia prior to having their limbs pinched and being set on surgical plates. Prior to utilizing a scalpel to make an incision to expose the trachea, the neck area was cleaned with 70% alcohol. A cotton thread was inserted under the trachea after being exposed by pincers. The center of the exposed trachea was precisely poked. The trachea was stabilised after a 0.5 cm catheter was placed by wrapping the trachea around the catheter using the cotton thread. The catheter was used to carefully inject a 1ml filled phosphate buffered

saline (PBS) syringe into the lung. While gently rubbing the mice's thorax, the solution was aspirated. The BALF collected was placed into a 2ml sterile Eppendorf tubes after the syringe was removed from the needle. The collected BALF was then placed on ice before analysis.

Preparation of lung homogenate

Each mouse's lungs were carefully removed, immediately rinsed in 1.15% potassium chloride, and then homogenised in 5 mL of 0.1 M phosphate buffer pH 7.4. For the biochemical and molecular tests, the post mitochondrial supernatant was obtained by centrifuging the homogenates at 10,000 x g for 15 min at 4°C.

Bronchoalveolar lavage fluid cell count

The bronchoalveolar lavage fluid from the mice was obtained, centrifuged for 10 minutes at 4000 rpm, and the supernatant was stored at - 20°C. The cell pellet was re-suspended in 500 mL of phosphate buffered saline in accordance with Southam's (2007) methodology for total cell counts. Differential cell counts were performed using 70 µl of the re-suspended pellets stained with the HEMA3 stain kit (Fisher ScientificTM).

Assay of oxidative imbalance biomarkers of redox state

As reported by Sun and Zigman (1978), the activity of superoxide dismutase (SOD) in tissue homogenate was analysed. The inhibition of epinephrine autooxidation was observed in alkaline media (pH 10.2). The amount of SOD required to generate a 50% suppression of the oxidation of adrenaline to adrenochrome over the period of one minute is equal to one unit of SOD activity. According to Claiborne's (1985) approach, the catalase (CAT) activity in tissue homogenate was measured. The assay mixture included 0.1 mL of tissue homogenate supernatant, 1.9 mL of 50 mM phosphate buffer, and 1 mL of 30 mM H_2O_2. Continuous monitoring of H_2O_2 oxidation was done for 60 s using spectrophotometric measurements at 240 nm. The difference in absorbance, which was represented as unit/mg of protein, was used to calculate the CAT activity. The amount of reduced glutathione (GSH) in the lung tissue homogenate was assessed using the method described by Jollow et al. (1974). The reaction mixture comprises of 1500 mL of 4% sulphosalicyclic acid, 900 mL of distilled water, and 100 mL of lung homogenate. The reaction mixture was centrifuged for 10 minutes at 4000 rpm after being left to stand for 5 minutes. A chromophoric molecule with a molar absorbance at 412 nm was produced by mixing 500 mL of supernatant, 2000 mL of 0.1M phosphate buffer (pH 7.4), and 250 mL of Ellman's reagent. Habig et al. (1974) method was used to measure glutathione-S-transferase (GST) activity. The assay combination contained 30 µL of reduced GSH (0.1 M), 150 µL of 2,4-dinitrochlorobenzene (CDNB) (3.37 mg/mL), 2.79 mL of phosphate buffer (0.1 M, pH 6.5), and 30 L of sample. Before measuring the absorbance at 340 nm in relation to the blank, the reaction was let 60 seconds to stabilize. The amount of ascorbic acid (AA) in tissue homogenates was determined using the technique described by Jagota and Dani (1982). The Folin-Ciocalteu method results in a blue colour with a maximum absorbance at 760 nm as AA in biological samples interacts with the reagent. To measure the concentration of thiobarbituric acid reactive species in tissue homogenates, Varshney and Kale's (1990) approach was applied. The process involved the combination of thiobarbituric acid and malondialdehyde (MDA) to produce a persistent pink chromophore with a maximum absorbance of 532 nm (PG instruments t60 UV/VIS spectrophotometer). The reaction mixture constituted 400 µL lung homogenate, 1600 µL TRIS-KCl buffer (pH 7.4), 500 µL 30% TCA, 500 µL 0.75% TBA. The tubes were placed on shaking water bath at 90-95 °C for 1 hour. Tubes were cooled on ice and centrifuged at 4000rpm for 10 minutes.

Assay of inflammation biomarkers

According to the Green et al. (1982) approach, the nitrite level was estimated to determine the nitric oxide (NO) concentration. Using nitrite, one of the stable oxidation products of NO is measured. A pink azo colour is produced when nitrite combines with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Using its absorbance at 550 nM, the azo dye generated in this assay is quantified spectrophotometrically. It was determined that myeloperoxidase's spectrophotometric activity could be measured using the technique outlined by Kim (2012). A plate was filled with 10 µL of the sample, 80 µL of 0.75 mM H2O2, and 110 µL of the TMB solution (2.9 mM TMB in 14.5% DMSO and 150 mM sodium phosphate buffer at pH 5.4). The plate was then incubated at 37°C for 5 minutes. After adding 50 L of 2M H2SO4, the reaction was stopped, and the myeloperoxidase (MPO) activity was calculated by calculating the absorbance at 450nm (PG instruments t60 UV/VIS spectrophotometer). MPO activity was calculated at mg/tissue.

Histopathological analysis

Lung tissues that had been paraffin-embedded were sectioned to a thickness of 5 µm, deparaffinized, and stained with haematoxylin and eosin. According to the procedure outlined by Fisher et al. (2008), the

microanatomy of lung tissue was evaluated with a light microscope (Nikon Diaphot, USA) (400 magnification). Histopathological examination and explanations were done by a pathologist.

Quantitative quantification of histology

Histological scoring was carried out following Hematoxylin and eosin (H & E) staining and shown in Figure 7. A semi quantitative morphometric analysis scoring system was used to analyze the lung injury and the scoring criteria are as follows. 0: Normal appearance; 1: Mild interstitial hyperemia, polymorphonuclear leukocyte infiltration; 2: Paravascular edema and moderate pulmonary structural damage; 3: Massive cell infiltration and moderate alveolar structure destruction; 4: Massive cell infiltration and severe lung structural damage (Oishi et al., 2012). All the histological studies were performed in a blinded fashion. Quantitative quantification of histology was done by a pathologist.

Statistical analysis

The results were given as mean ±S.D. Data were analysed using the Tukey post hoc test and an ANOVA using Graphpad Prism (V 8.01). P values of 0.05 or below were regarded as statistically significant.

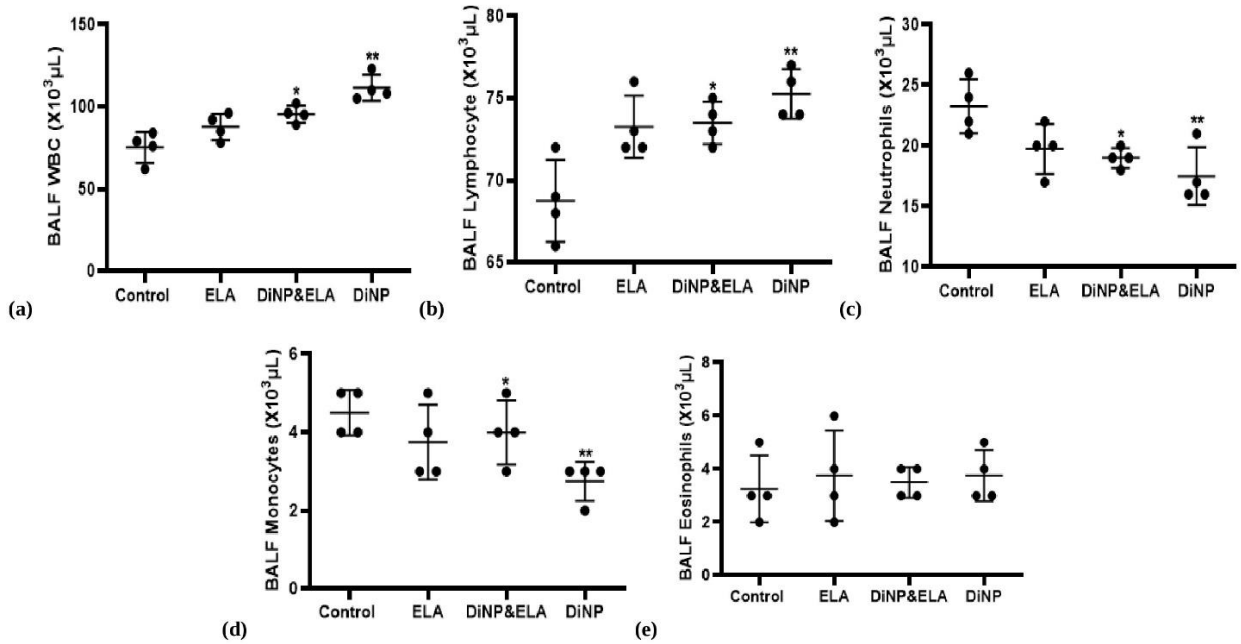
RESULTS

Ellagic acid suppresses total and differential cell count of DiNP-induced asthma in mice

In the bronchoalveolar lavage fluid, administration of diisononyl phthalate (DiNP) alone resulted in a substantial ($p < 0.05$) increase in lymphocytes (10%) and leukocytes (67%) and a significant decrease in neutrophils (31%) and monocytes (61%) compared to the control group (Figure 4). Eosinophil cell count showed no discernible difference. When compared to mice exposed to DiNP alone, co-administration of ELA with DiNP reduced DiNP's ability to cause an increase in lymphocytes (5%), leukocytes (16%), as well as a decrease in neutrophils (15%) and monocytes (27%) ($p < 0.05$).

Figure 4

Bronchoalveolar Lavage Fluid Total Cell Count



Note. Bronchoalveolar lavage fluid total cell count (a) WBC, (b) lymphocytes, (c) Neutrophils, (d) Monocytes, and (d) Eosinophils levels. The values are expressed as Mean ± SD of four mice (n = 4) in each group. WBC= White blood cells; BALF= Bronchoalveolar lavage fluid. *Significant difference ($p < 0.05$) between DiNP and DiNP&ELA. ** Significant difference ($p < 0.05$) between control and DINP.

Ellagic acid ameliorates DiNP-induced alterations in antioxidant status in asthmatic mice

Figure 5 depicts the protective effect of ELA against DiNP-induced modifications in both enzymatic (CAT, SOD

and GST) and non-enzymatic (GSH and AA) antioxidants. When compared to control mice, exposure to DiNP alone caused a substantial decline in CAT (68%), GST (55%) SOD (20%), and GSH (92%) and AA (50%) concentrations, as well as a concurrent rise in MDA (92%) levels ($p < 0.05$). Nevertheless, AA (72%) and GSH (10%) concentrations were elevated in the mice co-administered with ELA and DiNP along with increased CAT (32%), SOD (70%) and GST (65%) activity, whereas MDA (64%) levels were seen to decrease when compared to the DiNP only treated mice ($p < 0.05$). Furthermore, ELA increased AA concentration and GST activity by 74% and 58% respectively relative to the control group.

Ellagic acid ameliorates DiNP-induced inflammation in asthmatic mice

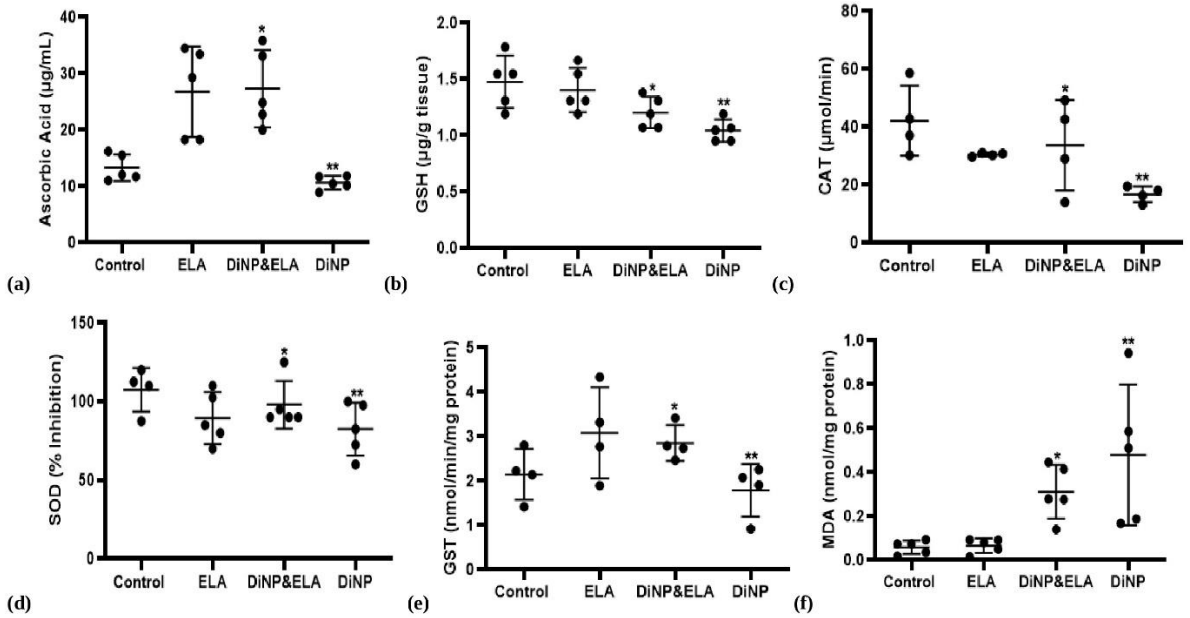
The protective effect of ELA against DiNP-induced changes in NO levels and MPO activity is seen in Figure 6. When compared to the control mice, there was a substantial ($p < 0.05$) increase in the amount of NO (88%) and MPO (66%) activity in the mice treated to DiNP alone. However, when compared to mice treated with DiNP alone, co-treatment with ELA with DiNP markedly reduced the amount of NO (55%) and MPO (34%) activity.

Ellagic acid prevented histoarchitecture of the lungs in DiNP-induced asthma in mice

The effect of DiNP on lung histoarchitecture and ameliorative role ELA is shown in Figure 7. Control mice in Figure 7 showed intact morphological features of respiratory epithelium comprising of the terminal bronchiole, leading into the alveoli duct. The terminal bronchiole (TB) of mouse lungs leads into the alveolar duct (AD) from where it branches into the alveoli sacs and terminate in the alveoli. The terminal bronchiole is seen consisting of simple cuboidal epithelium and a layer of smooth muscle fiber. This cuboidal ciliated epithelium of the terminal bronchiole loses it cilia distally as it becomes low cuboidal running into a less luminal diameter alveoli ducts from where it terminates into the alveoli predominantly lined by flattened type I cells and cuboidal type II cells. Also notable within the respiratory epithelium are few resident macrophages (megakaryocyte). However, DiNP-treated (group D) mice showed with varying degree of lesions such as distorted parenchyma, increase alveoli thickness, lymphocyte and neutrophils infiltration, fibrin deposition, pulmonary emphysema, pneumonia, intra alveolar hemorrhage and edema. The DiNP&ELA group (Group C) Histological observations reveals a reduction to complete absence in the observed lesions when compared with the DiNP-treated mice.

Figure 5

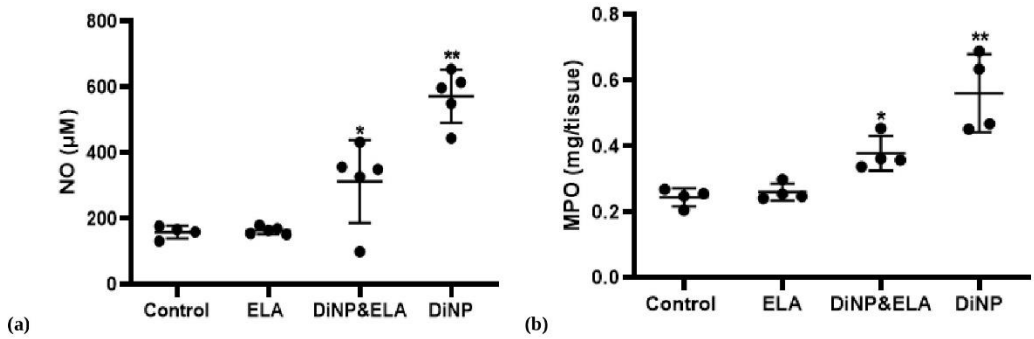
Pulmonary Antioxidants



Note. Pulmonary antioxidants (a) Ascorbic acid; (b) Reduced glutathione (GSH) levels; (c) Catalase (CAT); (d) Superoxide dismutase (SOD); (e) Glutathione-S-transferase (GST); and oxidative stress biomarker (f) malondialdehyde (MDA) level. The values are expressed as Mean ± SD of 4-6 mice (n = 4-6) in each group. *Significant difference ($p < 0.05$) between DiNP and DiNP&ELA. ** Significant difference ($p < 0.05$) between control and DINP.

Figure 6

Pulmonary Inflammatory Activities



Note. Pulmonary inflammatory (a) Nitric oxide (NO) level; and (b) Myeloperoxidase (MPO) activity. The values are expressed as Mean ± SD of four mice (n = 4) in each group. *Significant difference ($p < 0.05$) between DiNP and DiNP&ELA. ** Significant difference ($p < 0.05$) between control and DINP.

Ellagic acid ameliorates DiNP-induced aberrations in histological scoring

The considered parameters used to assessed lung injury (i.e. Normal appearance; 1: Mild interstitial hyperemia, polymorphonuclear leukocyte infiltration; 2: Paravascular edema and moderate pulmonary structural damage; 3: Massive cell infiltration and moderate alveolar structure destruction; and 4: Massive cell infiltration and severe lung structural damage) were significantly increased ($P< 0.05$) in the DiNP-treated group (4.0+ 0.0) when compared with the control (0.42 ± 0.20). Also, there was significant difference when ELA&DiNP (2.0±0.14) treated groups when compared DiNP groups as shown in Figure 8.

DISCUSSION

In asthmatic circumstances, different cells are recruited and activated, these cells and the mediators they release all contribute to the complex inflammatory process of airway inflammation. Eosinophils, mast cells, and lymphocytes, however, are the main factors causing this inflammation. The production of mucus plugs, which are composed of proteins from airway arteries and airway epithelial cells, causes the lumen of inflamed airways to become narrow and edematous (Wang et al., 2018). Ellagic acid has been demonstrated to possess antiallergic, anti-inflammatory, and pneumoprotective effects (García-Nino and Zazueta, 2015). The effect of ELA on pulmonary inflammation, oxidative stress and lung histoarchitecture in DiNP-induced asthma in mice was investigated in this study.

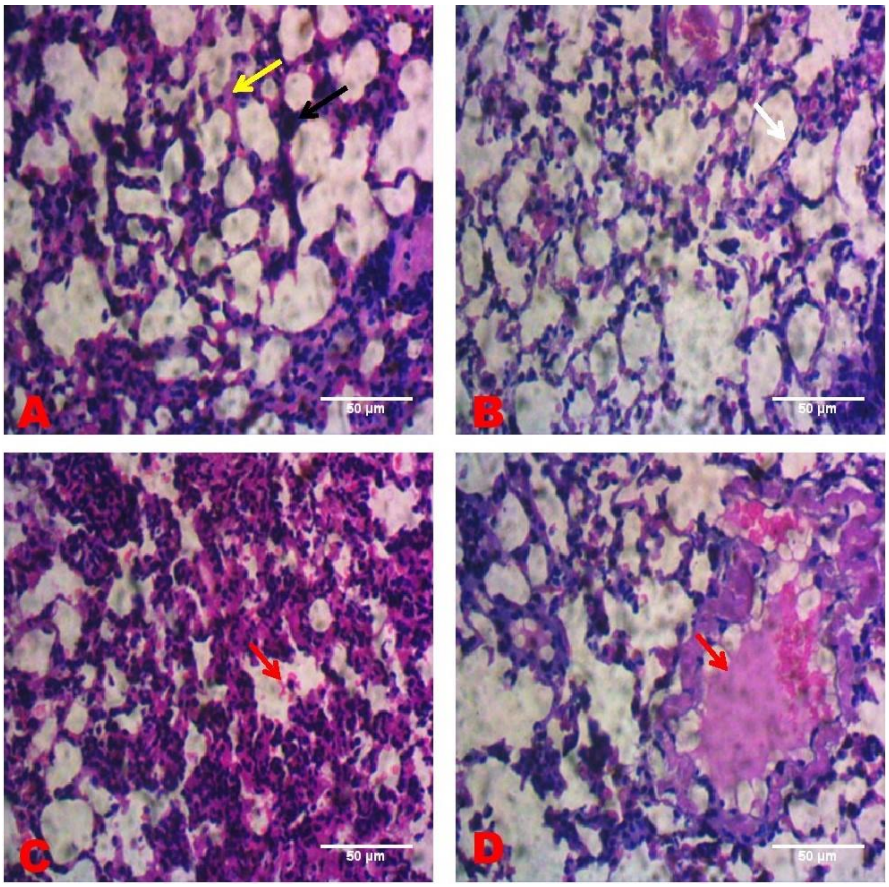
Inflammatory cells such as eosinophils, neutrophils, and lymphocytes are involved in airway inflammation (Elieh et al., 2019). Inflammation can be detected by an elevated total leucocyte count in bronchoalveolar lavage fluid. According to the study's findings, mice treated with DiNP had higher levels of leucocytes, and lymphocytes as well as lower levels of neutrophils and monocytes with no statistical difference in the levels of eosinophils. The increase is ascribed to the trans epithelial exudation of inflammatory cells into the BALF, which in mice treated with DiNP results in airway inflammation. When compared to mice treated with DiNP, administration of ELA with DiNP decreased the number of leucocytes, eosinophils, and lymphocytes while increasing the number of neutrophils and monocytes. The primary inflammatory cells implicated in allergy disorders are eosinophils. Eosinophils are not often found in healthy lungs, but their buildup in the lungs indicates an airway inflammation present in mild to severe forms of asthma (Rayees & Din, 2021). Reactive oxygen species such as hypobromite, superoxide, and peroxide are released by eosinophils, along with other cationic proteins such as eosinophil peroxidase. Eosinophil builds up in the lungs results in inflammation of the upper and lower airways. They are known to emit strong pro-inflammatory substances, particularly eosinophil peroxidase and leukotrienes, which are known to remodel the epithelial membrane in asthmatic airways (Rayees & Din, 2021). Neutrophils are one of the first innate immune cells to be recruited into the lungs after specific asthma-related events such allergenic challenges, virus-induced asthma exacerbations, or nocturnal crises, even though they are not always present in the airways of allergic asthmatic patients (Ramirez-Velazquez et al., 2013; Ilmarinen et al., 2015). Moreover, airway exposure to clinically relevant allergens that incite the features of allergic asthma in mice is associated with neutrophil migration into the airways (Radermecker et al., 2018). It has been shown that patients with severe asthma have neutrophil levels that are greater than normal in their airway lavage, but patients with mild and moderate asthma do not have elevated neutrophil levels in their airway discharges (Fahy et al., 2009). Given that the results of the current study and the one previously showed that the mice treated with DiNP had less

neutrophils, this may indicate that DiNP may cause mild to moderate asthma. Hence the low levels of neutrophils seen in DiNP-treated group. The pathogenesis of asthma is heavily influenced by the lymphocytes. Eosinophils in the airways are drawn in and stimulated, and plasma cells produce more IgE as a result. This is similar with the findings of the study, as mice treated with DiNP had higher levels of lymphocytes (Lin et al., 2013). The co-administration of ELA and DiNP, however, mitigated the observed increase.

Oxidants can cause airway hyperresponsiveness and inflammation. In asthmatics, there is an increase in the formation of reactive oxygen species by neutrophils, eosinophils, and macrophages, which results in a rise in hydrogen peroxide. Asthma development and progression is accompanied with oxidant/antioxidant imbalance caused by these reactive oxygen species migrating to the lungs (Silveira et al., 2019; Barnes, 2020). Both the development of asthma in humans and in lab animals is accompanied by the rapid generation of reactive oxygen species (ROS), which has an impact on the airway's structure and functionality (Sahiner et al., 2018). According to the study's findings, mice treated with DiNP had lower levels of both enzymatic (CAT and GST, SOD) and non-enzymatic (GSH and AA) antioxidants. This decline is linked to either an imbalance between the antioxidant-oxidant localized in the lungs of DiNP-treated mice or the excessive generation of ROS. However, relative to the DiNP-treated group, co-administration of ELA with DiNP dramatically halted the decline in levels and activity of the enzymatic and non-enzymatic antioxidants. On the other hand, lipid peroxidation refers to the oxidative breakdown of lipids. MDA, a by-product of lipid peroxidation, has been utilised in a number of biochemical assays to measure the degree of oxidative damage in asthma (Tang et al., 2021). The results of this investigation demonstrate that administration of DiNP led to a noticeably higher level of MDA. A higher degree of lipid peroxidation and a depletion of antioxidants, which leads to a propensity of the lung membrane to lipid oxidation, are shown by an increased level of MDA in the lung of the DiNP-treated group. However, the elevated level of MDA was able to be reduced by the administration of ELA and DiNP.

Figure 7

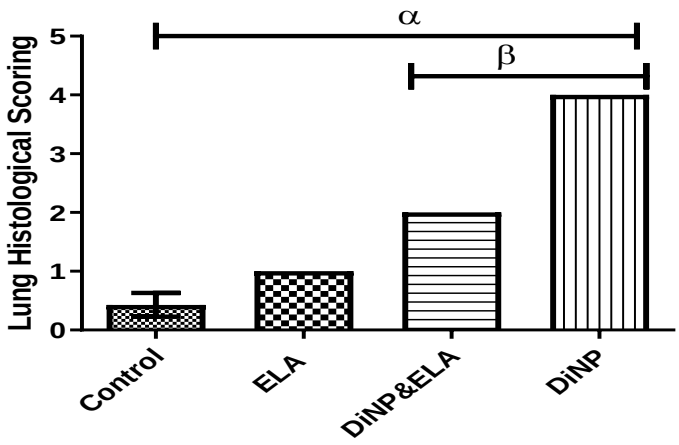
Representative Photomicrographs of Lung Sections Subjected to Hematoxylin and Eosin (H&E) Stain



Note. Representative photomicrographs of Lung sections subjected to Hematoxylin and Eosin (H&E) stain (x400). A- Control, B- ELA, C- ELA&DiNP, D- DiNP. Yellow arrow- smooth muscle, Red arrow – severe oedema, congestion and distorted respiratory epithelium, White arrow- intact respiratory epithelium, Black arrow-megakaryocyte, Scale bar- 50µm.

Figure 8

Histological Scoring of Lungs Pathology Following Hematoxylin and Eosin (H and E) Staining



Note. Histological scoring of lungs pathology following hematoxylin and eosin (H and E) staining. Values are expressed as mean ± SD, α- significantly different from control ($P < 0.05$), β- DiNP&ELA significantly different from DiNP ($P < 0.05$).

The respiratory host's defence mechanism increases NO generation. High NO levels can harm the respiratory system, which in turn affects the pathophysiology of inflammatory airway illnesses like asthma (Maruthamuthu et al., 2020). It has been demonstrated that an increase in NO occurs along with eosinophilic inflammation and is connected with other inflammation-related indicators in asthma (Qu et al., 2018). Myeloperoxidase (MPO) is a lysosomal enzyme that is specific to neutrophils and monocytes. It is found in the azurophilic granules of polymorphonuclear cells and oxidises a range of aromatic chemicals to provide substrate radicals for bacterial action. Additionally, MPO oxidises chloride ions to form the potent non-radical oxidant hypochlorous acid, which excessive synthesis leads to oxidative stress and tissue damage and is a sign of inflammation caused by polymorphonuclear leukocytes (Ndreppa, 2019). In this study, mice treated with DiNP had higher levels of NO and MPO activity in their lungs. Compared to mice treated with DiNP, administration of ELA dramatically lowered the elevated NO level and inhibited MPO activity. Here, ELA demonstrate a significant role in modifying the interaction of inflammatory mediators and suppressing neutrophilic inflammation.

Healthy individuals have a lung structural feature which include a normal airway, normal airway wall and relaxed airway smooth muscle, but the airways of asthmatic patients, upon exposure to allergens or asthma triggers constrict. There is overexpression of mucus, inflamed with swollen walls and tightened smooth muscle (Shastri et al., 2021). From this study, DiNP produced airway inflammation histologically by inflammatory cell infiltration, oedema, haemorrhage, and constrained alveoli space. Additionally, ELA ameliorated the aforementioned structural change and normalized lung shape in the ELA&DiNP-treated group.

CONCLUSION

According to this study's findings, ellagic acid decreases DiNP-induced asthma through the lungs' improved histoarchitecture, antioxidant, and anti-inflammatory properties. Ellagic acid may have therapeutic promise for treating asthma caused by DiNP or may offer hope for treating asthma caused by phthalates more generally.

AUTHOR CONTRIBUTIONS

Abosede Temitope Olajide originated the work and designed the experiment. Ebenezer Tunde Olayinka, Ayokanmi Ore and Samuel Abiodun Kehinde assisted with the experimental design and review of the manuscript. Cynthia Chisom Okoye and Abosede Temitope Olajide carried out the assays and analysed the data. Abosede Temitope Olajide developed the manuscript and edited the first draft.

ETHICS APPROVAL

Ethical Review Committee of the Faculty of Natural Sciences at Ajayi Crowther University approved the study's experimental design (FNS/ERC/23/001E).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest in this work.

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