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RESEARCH

Dysfunctional cardiac energy transduction, mitochondrial oxidative stress, oncogenic and apoptotic signaling in DiNP-induced asthma in murine model

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Abstract
Diisononyl phthalate (DiNP) has been associated with the development of allergies, asthma, and allergic airway inflammation. Through a complex interplay of signals and feedback mechanisms, the lungs communicate with the heart to ensure maintenance of homeostasis and supporting the body's metabolic demands. In the current study, we assessed the crosstalk between DiNP-induced asthma and cardiac cellular respiration, oxidative stress, apoptotic potential, and induction of oncogenic factors. Ten male BALB/c mice with a weight range of 20–30 g were divided into two groups, each comprising five mice. Group 1 (control), was administered saline orally for a duration of 30 days. In contrast, group 2 (DiNP group), received 50 mg/kg of DiNP to induce asthma. After the final administration and asthma induction, the mice were euthanized, and their hearts were excised, processed, and subjected to biochemical analyses. The DiNP group had downregulated ($P < 0.05$) activities of the enzymes of glycolysis, tricyclic acid cycle, and electron transport chain except the hexokinase and succinate dehydrogenase activity which were upregulate relative to control. Also, oxidative distress markers (GSH, CAT, and MDA and SOD) were also perturbed. Biomarkers of inflammation (MPO and NO) were considerably higher ($P < 0.05$) in the heart of DiNP-induced asthma mice as compared with the control group. Furthermore, DiNP-induced asthma group has an increased cardiac caspase-3, Bax, c-Myc and K-ras, and p53 while the Bcl2 decreased when compared with control. Overall, the findings indicate that DiNP-induced asthma impairs cardiac functions by induction of key cardiac oncogenes, downregulation of cardiac energy, transduction of enzymes, and promotion of oxidative stress and cellular death.

Keywords Mitochondria · Diisononyl phthalate · Glycolysis · Oxidative stress · Apoptosis · Asthma · Energy metabolism · Oxidative phosphorylation · Cellular respiration · Inflammation

Introduction

Over 300 million people worldwide suffer from asthma, a prevalent allergy condition that is on the increase. This condition causes the airways to constrict in response to specific stimuli, and it is typically treatable. The bronchi's smooth muscles constrict during an asthma attack, narrowing the bronchi (called bronchoconstriction), and this tightening is reversible. Shortness of breath coughing, chest tightness, and wheezing are some of the symptom's indicative of asthma in a clinical context (Bhatiaa et al. 2022). Continuous exposure to allergens such as environmental contaminants, cigarette smoking, viruses, stress, and air pollution raises the risk of asthma development. Asthma in older children and adults has also been related to environmental variables, such as exposure to dust mites, cockroaches, and pet dander, along with other environmental allergens (Milligan et al. 2016).

The lungs and the heart work together through a complex interplay of signals and feedback mechanisms. The respiratory system, centered in the lungs, facilitates the exchange of oxygen and carbon dioxide, while the circulatory system, anchored by the heart, pumps blood to deliver oxygen and nutrients throughout the body. One key aspect of cross talk is the regulation of blood oxygen levels. Inhaling causes the lungs to take in oxygen from the air, which the heart subsequently pumps to the body's tissues as oxygenated blood. At the same time, the heart receives signals from the body, including the levels of

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oxygen and carbon dioxide, which influence its rate and strength of contraction (Muir 2015).

The swift deterioration of symptoms and prompt enhancement in respiratory condition after initiating treatment are recognized as distinctive features of cardiac arrest resulting from asthma. It is suggested that asthma is infrequently identified as a cause of cardiac arrest. When asthma is active, a cardiovascular episode like stroke or heart attack can amplify the risk, and the consistent use of daily asthma medication may elevate that risk by 60% over a decade (Corlateanu et al. 2021). The connection between cardiac dysfunction and asthma may be attributed to inflammation, as elevated levels of inflammation are associated with both asthma and heart disease. Individuals with asthma often experience both allergic and non-allergic reactions, implying potential communication between molecular pathways related to allergies and those unrelated to allergies in various organs. Past research has established a correlation between asthma and a heightened likelihood of cardiovascular dysfunctions such as heart attack, heart disease, and stroke (Carter et al. 2019).

Phthalates, identified as environmental pollutants, find its application in various products such as food packaging, personal care items, nutritional supplements, children's toys, pharmaceuticals, insecticides, cleaning supplies, lubricants, paints, adhesives, and solvents (Haji et al. 2017). The occurrence of asthma and allergies has been associated with exposure to phthalates, as these endocrine-disrupting chemicals (EDCs) can directly induce inflammation in the airways (Dietert 2015). Specifically, exposure to DINP has been shown to stimulate allergic airway inflammation and perturb lung energy metabolism pathway in mice (Olajide et al. 2023; Kehinde et al. 2023).

With numerous studies demonstrating a connection between exposure to phthalates and cardiovascular diseases, only a small number of studies have linked animal cardiac dysfunctions caused by exposure to phthalates to those caused by DiNP. As a further baseline study for the safety evaluation of DiNP, the current study aims to investigate various perturbations orchestrated by DiNP-induced asthma via cardiac energy transduction, oxidative distress, apoptosis, and oncogene activation in cellular pathways.

Materials and methods

Test materials, chemicals, kits, and reagents

DiNP, which was procured from Relonchem Ltd., Chesire, UK, CYPRESS®Diagnostics, Belgium, supplied the lactate dehydrogenase test kit. The enzyme-linked immunosorbent assay (ELISA) kits for K-ras, caspase-3, c-Myc, Bcl-2, p53, and Bax were products of Cusabio Technology Llc, Houston, TX, USA. Other analytical grade chemicals and reagents were sourced from Sigma Chemical Co., USA, and Carlroth GMBH, Karlsruhe, Germany.

Animals

This investigation utilized ten (10) healthy male mice of 20–30 g in weight. The mice were sourced from the University of Ibadan (Department of Veterinary Anatomy). A 1-week acclimatization period preceded the commencement of the study, during which the mice were given standard laboratory chow and unlimited access to drinking water. They were housed under conditions featuring a natural photoperiod of 12 h of light and 12 h of darkness. The experimental protocol obtained approval from the Animal Ethical Review Committee of Ajayi Crowther University's Faculty of Natural Sciences (FNS/ERC/23/001E). Throughout the study, the mice received care adhering to the conditions stipulated in the "Guide for the Care and Use of Laboratory Animals" as per the guidelines from the National Academy of Science (NAS), as reported by the National Institute of Health (NRC 2011).

Experimental design

The male mice were divided into two (2) groups, each consisting of five (5) mice. The treatment protocol for each group was as follows: group 1 (control) received normal saline for a duration of 30 days, while group 2 (DiNP) mice were administered 50 mg/kg of DiNP (intranasal and intraperitoneal). The dosage of DiNP was determined based on findings from prior studies (Cornelio et al. 2013; Hwang et al. 2019; Kehinde et al. 2023). The mice received treatment in adherence to established guidelines for the care and handling of laboratory animals (NRC 2011). All mice were euthanized 24 h after the final administration.

Induction of asthma

The primary method employed for sensitizing the mice involved an intraperitoneal injection of DiNP (50 mg/kg) on day 0. Secondary sensitization of the mice occurred through intraperitoneal injections of DiNP (50 mg/kg) on days 3 and 10. Additionally, on days 19, 21, and 23, the mice underwent intranasal injections of 50 mg/kg of DiNP, which was diluted in 50 mL of saline. (see Fig. 1).

Sample collections and preparations

Following the final administration, the mice were euthanized, and the hearts were excised. The excised hearts were rinsed in 1.15% KCL (ice-cold). The heart was homogenized using a potter-elevehjem homogenizer in a 5% weight/volume solution of phosphate-buffered saline (0.1 M PBS; pH 7.4) after blotting and weighing. The homogenate underwent centrifugation at 12,000 × *g* (10 min at 4 °C). The supernatant obtained was utilized for subsequent biochemical assays.

Isolation of mitochondrial fraction

A protocol outlined by Liao et al. (2020) was used to isolate the mitochondria from the hearts of male mice.

Determination of glycolytic enzymes activities

The assessment of hexokinase activity was carried out using the procedure described by Colowick (1973). The measurement of PFK activity relied on the change in absorbance at 340 nm, employing established procedures as described by Sins and Blass (1986). Jagannathan et al. (1956) established protocol was utilized to evaluate aldolase catalytic activity. LDH activity was determined using the LDH Kit in accordance with the instruction of the manufacturer (CYPRESS).

Determination of enzyme activitiesof the TCA and ETC

A methodology outlined by Thorne (1962) was used to determine cardiac malate dehydrogenase (MDH) activity. The procedure described by Veeger et al. (1969) was employed to assess the activity of succinate dehydrogenase (SDH) in the heart. The spectrophotometric enzyme assay, as detailed by Srere (1969), served to determine citrate synthase (CS) activity. Romkina and Kiriukhin (2017) method was used to assess of isocitrate dehydrogenase (IDH) activity. The respiratory complexes (I, II, III, and IV) activities were spectrophotometrically determined at 340 nm in the mitochondria, using the protocol detailed by Medja et al. (2009).

Determination of antioxidant/oxidative stress markers

The determination of the cardiac lipid peroxidation marker (MDA) followed the method outlined by Buege and Aust (1978). Concentration of cardiac nitric oxide (NO) was estimated using Griess reagent, as outlined by Green et al. (1982). The spectrophotometric activity of cardiac myeloperoxidase was measured following the method outlined by Kim et al. (2012). Cardiac reduced glutathione (GSH) level was estimated using the method outlined by Moron et al. (1979). Cardiac glutathione S-transferase (GST) activity was evaluated with the method detailed by Habig et al. (1974). The activity of cardiac superoxide dismutase (SOD) was obtained using the protocol of Misra and Fridovich (1972), and the activity of cardiac catalase (CAT) was assessed following the method of Sinha (1972).

Estimations of cardiac levels of apoptotic factors (p53, caspase-3, c-Myc, Bax, Bcl-2, and Ras)

The procedures outlined in the commercially purchased Cusabio ELISA kits (Cusabio Technology Llc, HTX, USA) were adhered to for the estimation of Ras, caspase-3, c-Myc, p53, Bcl-2, and Bax levels.

Statistical analysis

The results were presented as mean ± standard deviation (S.D.). The data underwent analysis using a *t* test through Graphpad Prism (V 8.01). *P* values equal to or less than 0.05 was used to define statistical significance.

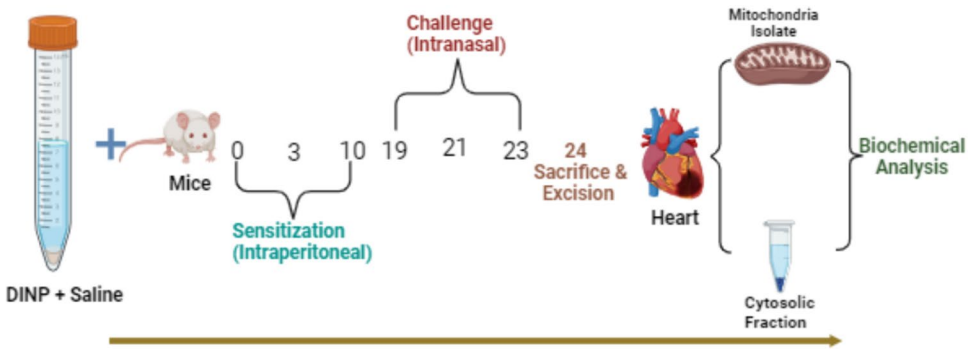

Fig. 1 Experimental design for DiNP-induced asthma (created in Biorender.com)

Results

DiNP-induced asthma perturbs activities of cardiac glycolytic enzyme in mice

Figure 2 shows how DiNP-induced asthma perturbs cardiac glycolytic enzyme activities in mice. The activities of cardiac PFK, ALD, and LDH were significantly ($P < 0.05$) decreased (26.58, 51.6, and 66.7% respectively) after induction of asthma with DiNP (50 mg/kg) compared to the control group. Nevertheless, there was a 59.1% increase in cardiac hexokinase (HK) activity upon the induction of asthma relative to the control group.

DiNP-induced asthma modulates cardiac oxidative phosphorylation enzyme activities in mice

Figure 3 illustrates the modulation of cardiac oxidative phosphorylation enzyme activities in DiNP-induced asthma. This modulation is typified by the inhibition of the activities of the enzymes involved in the cardiac tricarboxylic acid cycle, with significant decreases ($P < 0.05$) in MDH (73.9%), CS (60.03%), and IDH (43.03%). Notably, succinate dehydrogenase exhibited an upregulated activity of 74.4%. Additionally, respiratory chain enzymes (complexes I–IV) showed a general downregulation, with the exception of complex II, which experienced an upregulation relative to the control group. Specifically, respiratory complexes I, III, and IV activities were downregulated by 70.59%, 21.43%, and 97.12%, respectively, while complex II activity demonstrated 93.83% upregulation relative to the control.

DiNP-induced asthma induces cardiac oxidative distress in mice

Figure 4 indicates activities of cardiac oxidative biomarkers in DiNP-induced asthmatic rats. Cardiac enzymatic antioxidants—superoxide dismutase, catalase, and glutathione-s-transferase activities were significantly ($P < 0.05$) inhibited (56.0, 57.3, and 96.3% respectively). Cardiac non-enzymatic antioxidants—glutathione and ascorbic acid concentration were also decreased (75.04 and 69.62% respectively) while MDA activity was significantly ($P < 0.05$) increased (79.17%) relative to control.

DiNP-induced asthma stimulates cardiac inflammation in mice

Figure 5 indicates cardiac inflammation biomarker activity and level in DiNP-induced asthmatic mice. Cardiac nitric oxide (NO) concentration significantly ($P < 0.05$) increased (80.48%) in DiNP-induced asthma mice relative to control. Activity of cardiac myeloperoxidase (MPO) was also upregulated (51.72%) when compared with control.

DiNP-induced asthma alters cardiac oncogenic and apoptotic factor levels in mice

DiNP-induced asthma caused alterations in the levels of apoptotic and oncogenic in mice, as depicted in Fig. 6. Notably, DiNP-induced asthma resulted in a significant ($P < 0.05$) reduction in cardiac BCL-2 (74%) and an increase in cardiac c-Myc (59%), Cas-3 (65%), K-ras (82%), Bax (70%), and p53 (51%) levels compared to the control group.

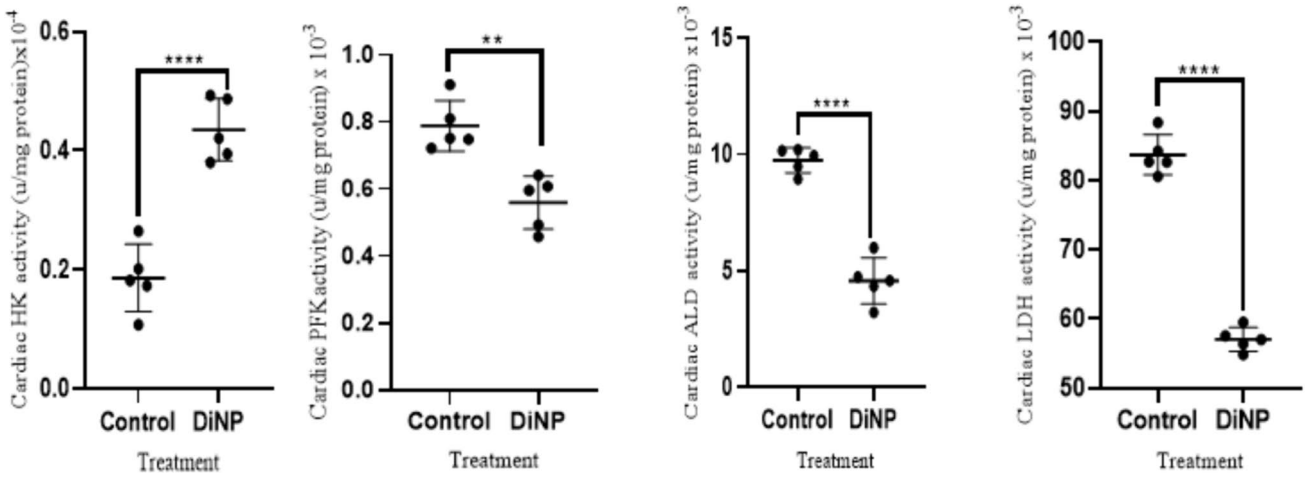

Fig. 2 Effect of DiNP-induced asthma on cardiac glycolytic enzyme activities in mice. The values represent the mean ± SD of five rats ($n = 5$) per group. * means significantly different ($P < 0.05$) relative to control. HK, hexokinase; PFK, phosphofructokinase; ALD, aldolase; LDH, lactate dehydrogenase

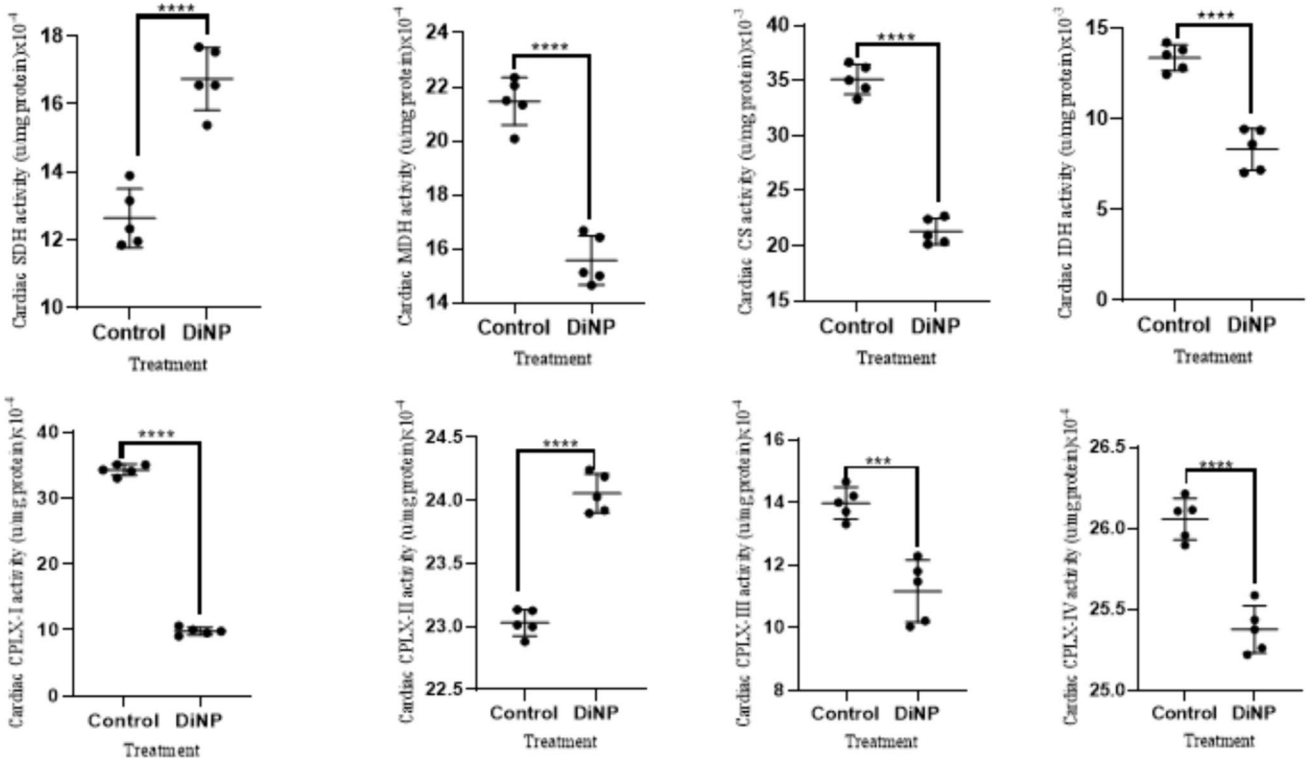

Fig. 3 Effect of DiNP-induced asthma on cardiac oxidative phosphorylation enzyme activities in mice. The values represent the mean ± SD of five rats ($n = 5$) per group. ∗ means significantly different ($P < 0.05$) relative to control. SDH, succinate dehydrogenase; MDH, malate dehydrogenase; IDH, isocitrate dehydrogenase; CS, citrate synthase; CPLX, respiratory chain complex

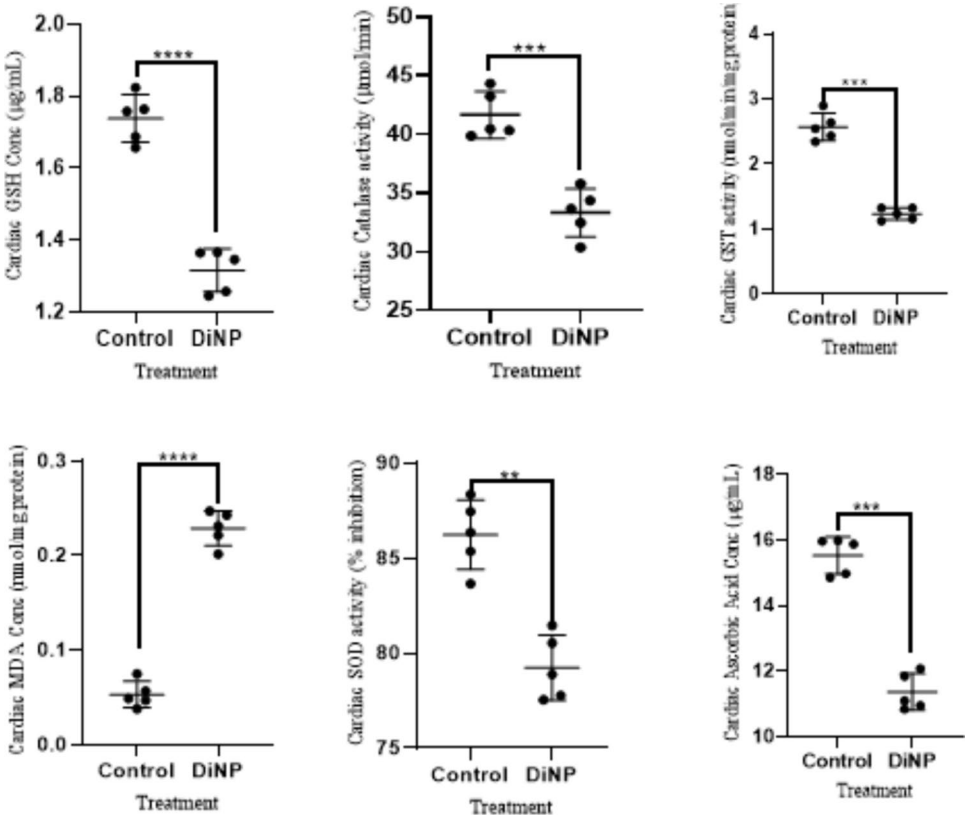

Fig. 4 Effect of DiNP-induced asthma on cardiac oxidative distress enzyme activities in mice. The values represent the mean ± SD of five rats ($n = 5$) per group. ∗ means significantly different ($P < 0.05$) relative to control. SOD, superoxide dismutase; GSH, glutathione; MDA, malondialdehyde; CAT, catalase; AA, ascorbic acid; GST, glutathione-s-transferase

Discussions

The heart and lungs are pivotal organs and their functions are closely intertwined. This connection arises not only from their shared location in the chest cavity but also from their collaborative processes. The heart plays a crucial role in pumping blood rhythmically throughout the body. This starts a cycle in which the pulmonary loop receives deoxygenated blood and sends it to the lungs where oxygen is replenished (Kim 2022). Subsequently, the systemic loop distributes the oxygen-enriched blood to other vital organs. However, asthma, a condition characterized by inflammation and thickening of the airways in the lungs, hampers the smooth exchange of oxygen, contributing to increased difficulty in breathing. This respiratory impairment can lead to elevated blood pressure, known as pulmonary hypertension, imposing strain on the heart's right side and potentially resulting to a heart failure (Rosenkranz et al. 2020). The present study employs mice as a model to unveil insights into the interplay and interdependence between the lungs and heart concerning asthma induced by DiNP. This research provides initial knowledge regarding the intricate relationship between these two vital organs in the context of DiNP-induced asthma.

The glycolytic pathway holds a significant role in the body's metabolic processes, representing a series of enzymatic reactions responsible for breaking down glucose (glycolysis) into pyruvate. This process generates essential energy sources, namely adenosine triphosphate-ATP and nicotinamide adenine dinucleotide-NADH. The metabolic activity of glucose in the heart is crucial for both normal physiological functioning and pathological conditions, underscoring its paramount importance (Tran and Wang 2019). In this investigation, it was noted that, except for hexokinase (HK) activity, the cardiac glycolytic enzymes—phosphofructokinase (PFK), aldolase (ALD), and lactate dehydrogenase

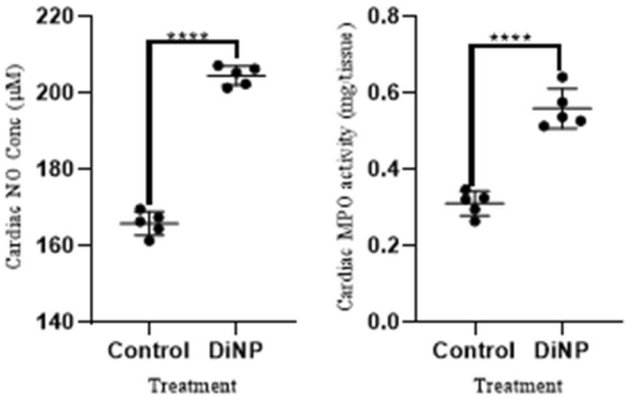

Fig. 5 Effect of DiNP-induced asthma on cardiac inflammatory biomarkers in mice. The values represent the mean ± SD of five rats ($n = 5$) per group. * means significantly different ($P < 0.05$)

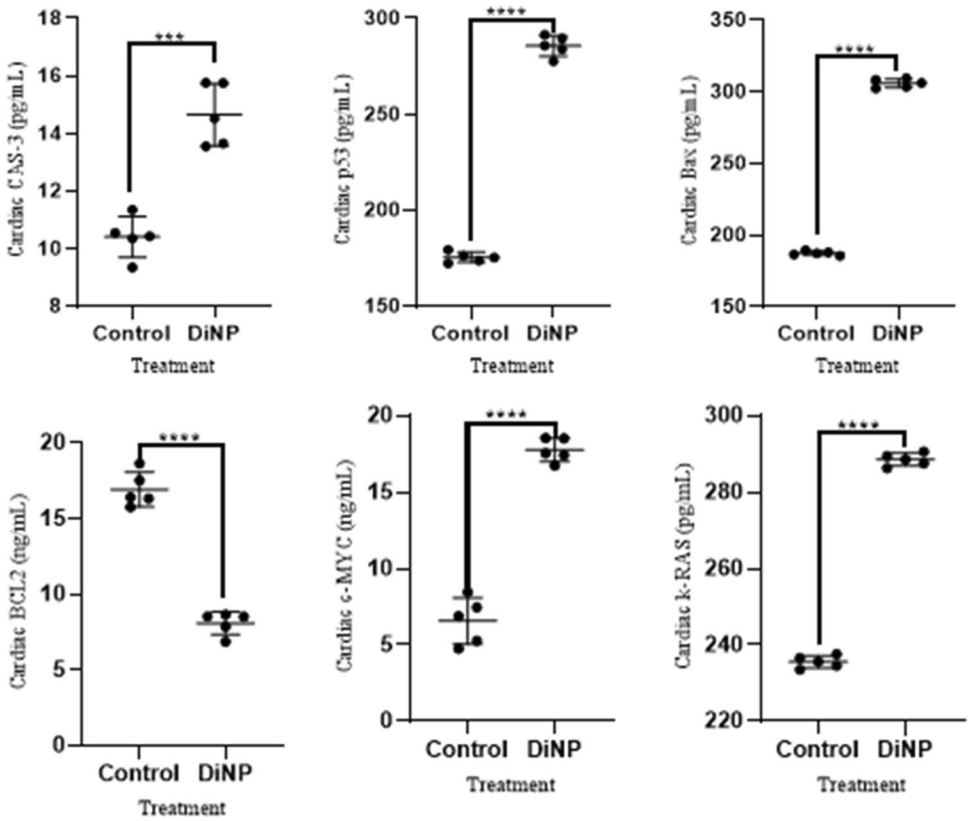

Fig. 6 Effect of DiNP-induced asthma on cardiac oncogenic and apoptotic. The values represent the mean ± SD of five rats ($n = 5$) per group. * means significantly different ($P < 0.05$) relative to control. Cas-3, caspase-3; K-ras, Kirsten-rat sarcoma virus; p53, tumor protein P53 factors; Bax, apoptosis regulator BAX; BCL-2, B-cell lymphoma 2; c-Myc, cellular myelocytomatosis

(LDH)—underwent downregulation in DiNP-induced asthmatic mice, in comparison to the control group.

This aforementioned observation suggests a potential deficiency in the ultimate product of glycolysis, pyruvate, which could impede ATP production in cardiac tissue. The observed increased activity of HK and subsequent inhibited activities of other glycolytic enzymes under study may be attributed to the fact that there are different hexokinase isozymes (I, II, III, and IV) in vertebrates, each with distinct properties and tissue-specific expression. Hexokinase IV, also known as glucokinase, is specific to the liver and pancreas. These isozymes allow differential control of glucose phosphorylation based on local conditions and physiological needs (Gupta and Gupta 2005). Furthermore, phosphofructokinase-1 (PFK-1), is a glycolytic enzyme that catalyze the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate. Despite increased hexokinase activity, PFK-1 can be inhibited due to several factors: high levels of ATP act as an allosteric inhibitor of PFK-1. When ATP is abundant, it signals that the cell has adequate energy, leading to PFK-1 inhibition. Conversely, AMP (adenosine monophosphate) activates PFK-1. Low ATP levels increase the AMP/ATP ratio, promoting glycolysis. An allosteric regulator, fructose 2,6-bisphosphate also stimulates PFK-1 activity. The balance between these regulators determines whether PFK-1 remains active or inhibited, regardless of hexokinase activity (Nare et al. 2023). Cardiac ALD on the other hand may have experienced activity inhibition due to acidic conditions (that is, there might have been an alteration in the cardiac pH conditions) and substrate availability, if fructose 1,6-bisphosphate is scarce; aldolase activity decreases. Furthermore, LDH activity inhibition can be attributed to NADH/NAD + ratio, in which high NADH levels inhibit the enzyme. In addition, acidic conditions affect its activity (Webb et al. 2017).

Furthermore, enzymes in the subsequent pathways (TCA and oxidative phosphorylation—OXPHOS), which typically contribute to energy generation in the heart, experienced downregulation, with some exceptions noted, including succinate dehydrogenase and respiratory complex II. SDH is a key enzyme in the TCA cycle that catalyzes the enzymatic conversion of succinate to fumarate while simultaneously transferring electrons to the ETC. SDH is distinct due to its embedding in the inner mitochondrial membrane and direct involvement in the TCA cycle and ETC. Increased SDH activity observed can have several implications which may be an enhanced electron transfer to the ETC that contributes to ATP synthesis, or elevated levels of fumarate, which can serve as a precursor for other metabolic pathways, and potential modulation of cellular redox balance (Nastasi et al. 2021). However, CS, IDH, and MDH had an inhibited activity, and this may cause the cardiac cells to prioritize succinate oxidation via SDH to maintain energy production, and dysregulation of TCA cycle enzymes can impact cellular redox state, metabolite levels, and overall metabolic flux (Gasmi et al. 2021).

Complex I, also known as NADH dehydrogenase receives electrons from NADH which are then transferred to ubiquinone (Q). In certain conditions (such as ischemia–reperfusion injury), complex I can become overwhelmed by a sudden influx of electrons, leading to reduced ubiquinone (Q) levels. This reduction in Q availability forces electrons to flow backward from ubiquinone to complex I, resulting in leakage of electrons to oxygen. This can account for the observed decreased activity of cardiac CMP I in DiNP-induced asthmatic mice. This process generates harmful reactive oxygen species (ROS) (Goetzman et al. 2023). Complex III (cytochrome bc1 complex) pumps protons across the mitochondrial membrane and transfers electrons to cytochrome c. During oxidative stress or mitochondrial dysfunction, complex III activity can be impaired, leading to decreased proton pumping and disrupted electron flow. Reduced complex III function may contribute to ROS production and cellular damage (Nolfi-Donegan et al. 2020). Using electrons from cytochrome c, complex IV (cytochrome c oxidase) converts molecular oxygen (O_2) to water (H_2O).

Factors such as hypoxia or toxins (like cyanide) can inhibit complex IV, disrupting the final step of ETC. Reduced complex IV activity affects ATP production and can lead to cellular dysfunction. Complex II (succinate dehydrogenase) directly receives electrons from $FADH_2$ (generated during the citric acid cycle). Unlike complex I, complex II bypasses the initial proton-pumping step. Increased cardiac complex II activity understudy may have occurred due to an excess of $FADH_2$ (e.g., due to high succinate levels). However, complex II does not contribute significantly to proton pumping, so it does not fully compensate for reduced activity in other complexes (Goetzman et al. 2023).

Furthermore, it has been established that the disruption of the cardiac electron transport chain (ETC) function elevates cardiac intracellular reactive oxygen species (ROS) levels (Chen and Zweier 2014). Complex III stands out as the principal location for the overall net generation ROS in mitochondria, hindering that the flow of electrons into complex III efficiently hinders ROS production within these organelles. The prominence of complex III in ROS generation is linked to the diversion of ROS products away from the antioxidant defenses in the matrix. Interestingly, the inhibition of cytochrome oxidase amplifies ROS production, mainly originating from specific sites upstream in the electron transport chain, with a noteworthy emphasis on the upregulated succinate dehydrogenase. These ROS play a pivotal role in regulating diverse physiological processes, encompassing inflammation, migration, contraction/expansion, apoptosis, cell growth, and fibrosis. Within the cardiovascular system,

hydrogen peroxide, superoxide anions, reactive nitrogen species, hydroxyl radicals, peroxynitrite, and nitric oxide (NO), collectively serve significant biological functions (Vara and Pula 2014). NADPH oxidase 2 (NOX2), a primary source of ROS in the heart, undergoes activation following myocardial infarction (MI), resulting to the substantial generation of ROS. Following myocardial infarction, increased ROS generation plays a role in ventricular remodeling and the emergence of heart failure. There is increasing evidence that excessive ROS play a role in the development and progression of heart pathologies. Examination of the results reveals reduced activities of enzymatic antioxidants (GST, SOD, and CAT), along with a decline of non-enzymatic antioxidants (AA and GSH) concentrations and an elevated level of malondialdehyde (MDA). These results collectively indicate the occurrence of oxidative stress in the heart orchestrated by DiNP-induced asthma.

In addition, inflammation serves as a responsive mechanism to harmful stimuli, encompassing infection or tissue injury, and entails the regulated mobilization of blood components, such as plasma and leukocytes, to the affected site (Abdulkhaleq et al. 2018). While a controlled inflammatory response is typically deemed beneficial, offering protection against infection, it may turn detrimental if unregulated. Notably, DiNP-induced asthma in mice exhibited a significant rise in inflammatory biomarkers (NO and MPO), unequivocally indicating the interconnectedness between DiNP-induced asthma and cardiac inflammation.

Apoptosis, a pivotal process of programmed cell death observed in both malignant and healthy tissues, induces the demise of mutated or damaged cells in healthy tissues, thereby thwarting the potential for future mutagenesis and cancer development. The findings of this study reveal an increase in Bax, Cas-3, c-Myc, p53, K-ras, and a decrease in BCL-2. Alterations in apoptosis can contribute to various clinical disorders, including malignancies. P53, a critical tumor suppressor protein, plays a pivotal role in apoptosis and cell cycle arrest in response to DNA damage and cellular injury. Under normal circumstances, the murine double-minute oncogene (MDM2) is the primary regulator of p53, which is present in the cytosol at low levels. However, upon DNA damage induced by factors like UV radiation or other environmental assaults, p53 accumulates in the nucleus, triggering cell cycle arrest and apoptosis to prevent mutations and the carcinogenesis (Gudkov and Komarova 2016). The Bcl-2 protein family, playing an important role in apoptosis, can either facilitate or hinder this process, primarily operating at the mitochondrial level. Categorized as pro- and anti-apoptotic members, these proteins exert their functions in connection with the outer mitochondrial membrane (OMM). One way that Bcl-2 works to prevent cell death is by inhibiting the permeabilization of the OMM and the ejection of proteins from the intermembrane gap. On the other hand, transcriptional or posttranscriptional pathways via BH3-only proteins like Bid, Puma, or Noxa control the activation of proapoptotic multidomain members like Bak and/or Bax. Membrane permeabilization, the release of proapoptotic proteins from the mitochondria, and eventual cell death follow the oligomerization and insertion of Bax/Bak into the OMM. Caspases-dependent or -independent pathways are activated by cells based on the specific proteins that are released from the intermembrane gap. By inhibiting apoptosis and interacting with pro-apoptotic proteins, Bcl-2 contributes to cell survival. Malignancies characterized by heightened Bcl-2 expression often exhibit unfavorable prognoses, and Bcl-2 protein level alterations play a pivotal role in the process of carcinogenesis (Czabotar et al. 2014).

Caspase-3 (a proapoptotic protease family) instigates nuclear modifications leading to apoptosis as reported by Boland et al. (2013). Although considered a favorable prognostic pointer for gastric malignancies, caspase-3 levels are diminished in various cancer types, like the breast and cervical cancer. Through the disruption of the mitochondrial membrane, c-Myc triggers programmed cell death and proapoptotic effectors such as holocytochrome C. It is possible that throughout this process, apoptosis-promoting Bcl-2 family members act as mediators for c-Myc (Juin et al. 2002).

It is well-established that Bax causes channel opening in mitochondria by interacting with the voltage-dependent anion channel (VDAC), which reduces membrane potential and releases cytochrome c. The tumor suppressor P53 regulates the expression of this gene, which is linked to P53-mediated apoptosis. It has been shown that c-Myc overexpression is connected to Bax activation (Annis et al. 2005). K-ras, involved in transmitting growth signals and mitogenic into the nucleolus and cytoplasm, plays a role in various ligand-mediated signal transduction pathways, influencing processes such as transformation, proliferation, differentiation, and cell death. In the present study, all apoptotic factors were significantly elevated in the DiNP-induced asthma group, while the level of Bcl-2 decreased compared to the control. It is interesting to note that while low levels of Bcl-2 may have adverse consequences, overexpression of Bcl-2 in some cancer cells can thwart pro-apoptotic signals, allowing cancer cells to thrive under conditions of stress. Bcl-2 promotes cancerous cells' ability to survive and resist medication, but it also offers potential for novel targeted therapeutics intended to eradicate cancerous cells (Tannoury et al. 2022). According to the study, there is an increase in apoptotic elements that may have a role in the development and progression of cancer.

Conclusion

In this investigation, alterations in cardiac cellular metabolism, inflammation, cardiac mitochondrial oxidative stress, and apoptotic factors were scrutinized in a mouse model of DiNP-induced asthma. The findings of this study propose that DiNP-induced asthma has adverse effects on cardiac mitochondria antioxidant status, leading to the activation of inflammatory markers. Additionally, it inhibits energy metabolism by impeding energy transduction enzymes, thereby hindering the synthesis and breakdown of ATP indicative of the heart not being able to produce sufficient ATP to fulfill its functions during a DiNP-induced asthma episode. These revelations have positioned mitochondria at the forefront of future research on cardiac-pulmonary crosstalk. Due to its critical role, high metabolic demand, and abundant mitochondrial content, the heart is particularly vulnerable to oxidative damage within the mitochondria. Consequently, the intertwined connectivity between pathologies of the lungs as having a resounding effect on the cardiac system has been established in DiNP-induced asthma potentiating several cardiomyopathies as related to mitochondria oxidative distress, inhibited cellular energy metabolism, and induction of apoptotic and oncogenic biomarkers necessitating the need for cardio-protective mechanisms to douse the adverse crosstalk effects of pulmonary and cardiomyopathies.

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Authors contributions S.A.K: Conceptualization, Methodology, Investigation, Data curation, Project administration, Resources, Supervision, Writing – original draft, review & editing. A.T.O: Formal analysis, Investigation, Resources, Methodology, Writing – Writing – review & editing. F.P.T: Resources, Formal Investigation, Writing-review and editing. DF: Software, Fund acquisition, Writing – review & editing, Resources N.R.H: Investigation, Methodology, Software, Writing – editing., Funding acquisition, A.M.E: Project administration, Writing – review & editing. A.S.J: Formal analysis, Investigation, Writing – Writing – review & editing. M.H.M.A: Methodology, Resources, Software. All authors revised and approved the submitted version of manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability The data obtained during the current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval The experimental protocol obtained approval from the Animal Ethical Review Committee of Ajayi Crowther University's Faculty of Natural Sciences (FNS/ERC/23/001E).

Competing interests The authors declare no competing interests.

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