Single and Combined Aluminium and Cadmium Exposure during Pregnancy Mediate Changes in Cardio Metabolic Indices in Mice

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ABSTRACT: The potential risk of Aluminium and Cadmium co-exposure is high owing to evidence of their co-contamination of several food products. Therefore, the present study investigated the effect of single and combined exposures of aluminium and cadmium during pregnancy on cardio metabolic changes in mice. Following delivery and at the end of 78 days postnatal development, it was observed that exposure to Al and Cd during pregnancy altered indices of cardiac function via pathways related to angiotensin, cardiac troponin and oxidative stress signalling which may have impacted directly on the histocstructural features of the heart. Comparatively, prenatal exposure to Cd alone impacted more negatively to the heart in relation to exposure to either Al only or co-exposure to Al and Cd.

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Heavy metals are non-biodegradable in nature; thus, they have an extended biological half-life (Gupta and Joia, 2016; Asagba et al., 2019). Exposure to these metals occurs through diverse human activities which include application of pesticides and fertilizers to the soil, sewage sludges, electroplating, mining and manufacturing, burning of fossil fuels, and the use of metallic cookware (Tasrina et al., 2015; Orisakwe et al. 2017; Quinteros et al. 2017; Olufemi et al., 2018; Obasi et al., 2019). When harmful metals seep into bodily tissues, other metals may be depleted as a result. These metals can then bioaccumulate and cause a variety of health problems, such as kidney damage, cancer, cardiovascular disease, mental retardation, growth retardation, lowered immune system, anaemia, abdominal pain, lipid peroxidation, and DNA damage, among other conditions. (Javed and Usman 2013; Asagba et al. 2019; Bazir et al. 2023; Khalaf et al. 2023). Although there are several heavy metals found in nature, Aluminium is very abundant and is ranked as the third most abundant element within the earth crust (Exley 2013; Gerard and Gray 2015). Pure aluminium is soft in nature and can combine with other metals and elements to form alloys which are harder. Such metals and elements include copper, silicon, manganese, zinc, magnesium and lithium. Aluminium metal and its alloys can be used in the production of cookware, doors, windows, roofing sheets, electrical equipment, including antacids, body care products, storage and packaging materials due to its affordability, light weight, high heat conductivity, malleability, and availability (Mohammad et al., 2011; Odularu et al., 2013; Alabi et al., 2020). In most developing countries, the recycling of aluminium-based materials is used for the production of forged aluminium cook wares from scrap metals like
computer parts, bicycles, lead batteries, old and broken spoons, cans and containers thrown in gutters. This singular process thus increases the exposure risk potential of aluminium as this often increases the propensity of aluminium leachates together with other toxic metals and metalloids into food (Yang et al. 2019; Ghobakhloo et al. 2024). There is increased evidence that the highest form of aluminium exposure comes through the food chain as the metal is primarily sourced from diets which include nuts, herbs, tea, spices, salt, grain products and processed cheese (Alabi and Yetunde, 2020). Also, the rampant usage of aluminium foils for the packaging of various meat and poultry products sauced with tomato sauce, salt, citric acid, vinegar and cooked in the oven at different temperatures, leads to the increase in aluminium exposure and its toxicity (Dordevic et al. 2019; Fermo et al. 2020, Covaliov et al. 2021). Nested between zinc (Zn) and mercury (Hg) in the periodic table of elements, cadmium (Cd) is a naturally occurring metal that exhibits chemical behaviour akin to that of zinc. It commonly exists as a cation with a divalent charge, attached with other elements (e.g., CdCl₂). Ingestion or inhalation are the main ways that humans are exposed to Cd. Based on particle size, 10–50% of cadmium dust inhaled is absorbed (Ichihi-Ifukor et al. 2013; Ezdom and Asagba, 2016; Asagba, 2019). In work environments, breathing in particulates from industrial exposure can be harmful. Soldering or welding, for instance, can result in a serious case of chemical pneumonitis. Consumption of tainted food (such as crabs, fish, organ meats, rice, leafy vegetables, etc.) or water (from outdated zinc/cadmium sealed water lines or industrial pollutants) might result in long-term health consequences from cadmium exposure (Horiguchi, 2020; Lien et al. 2021; Gao et al., 2022). Another possible cause of pollution is drugs and dietary supplement contamination. Adults who are exposed to Cd experience harm to numerous organ systems, including the nervous system (Zhao et al., 2015). Studies have demonstrated Cd to cross the placental barrier and affect foetal development (Dharmadasa et al., 2017). Reduced birth weights have occasionally been connected to prenatal blood lead levels (Luo et al., 2017). Previous studies have connected Cd to cerebral palsy (Hong et al., 2016). As a result, exposure to cadmium during lactation or pregnancy may be harmful to the baby, especially to the nervous system. The toxicity of Cd has been connected to numerous reasons, such as oxidative stress, cellular death, and inflammation that affects the kidney, testis, and brain (Rinaldi et al., 2017). Cd and Al have also been implicated in the remodelling of the heart structures and shape. Both metals are said to contribute to uncontrolled rise to hypertensive symptoms, clogged arteries, DNA oxidation and cardio mineral redistribution (Schmidt et al., 2016; Martins et al., 2021; Zhang et al., 2022; Ranke et al. 2023). While serum aluminium levels have been widely implicated in cardiac anomalies in patients undergoing dialysis, serum cadmium and cord blood cadmium levels is said to be significantly correlated with birth weight outcomes, cognitive development indices and risk of cardiac diseases (Oritsemuelebi et al., 2021). It is important to state therefore, that with the ubiquitous nature of these metals there exist risk potentials of their co-exposures among several inhabitants as research evidence reveals that these metals often occur side by side either in metal ores or as metalloids during recycling of different metallic products. Therefore, the objective of this study was to investigate the effect of single and combined exposures of aluminium and cadmium during pregnancy on cardio metabolic changes in mice offsprings.

MATERIALS AND METHODS

Chemicals and Reagents: CdCl₂ and AlCl₃ was purchased from sigma Aldrich UK while all other reagents were of analytical grade.

Ethical Approval: Ethical approval (REL/FOS/2023/05) for the study was granted by the Delta State University Faculty of Science Ethical committee in agreement with previously published guidelines by the World Medical Association (2000).

Acquisition of Animals for the Study: 40 neonate mice were used in this investigation. They were produced by breeding 20 adult non-pregnant female and 20 adult male mice at the age of 8–10 weeks. The animals were acquired from Delta State University's College of Medicine's animal facility in Abraka. The mice were given two weeks to acclimatize in the Department of Biochemistry. They were housed in well-constructed plastic mouse cages, with free access to food (hybrid growers' mash feed), water, and other supplies during the experiment similar to those reported by Mordi et al. (2021). The adult female mice's oestrous cycle and vaginal cyclic period were discovered towards the conclusion of the acclimation stage before one female and one male were placed in a mating cage, and a predetermined mating procedure was carried out.

Animal Treatment and Experimental Design: Day 1 following the mating process was determined to be the first day of pregnancy through the presence of spermatozoa following a vaginal smear. Different groups of pregnant mice were subjected to different treatments from gestation day 7 to 20. Group 1 was the control group (CTR) and received no treatment other than normal saline (0.9% NaCl) (10 ml/kg, PO). Group

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Collection and Preparation of Samples for Biochemical Assays: For the purpose of biochemical testing, six animals had their cervical discs removed. Blood was drawn via the aorta vein, and samples were centrifuged for 10 minutes at 4 °C at 10,000 rpm. After being separated into sterile, non-heparinized bottles, the serum samples were stored at -20 °C until they were needed to measure cardiac troponin I, angiotensin converting enzyme and creatinine kinase. Intermittently, the harvested cardiac tissues were homogenised at 3,500 rpm for three minutes in test tubes with 4 mL of 0.05M phosphate buffer (pH 7.4). Additionally, homogenates were spun in a cold centrifuge set to 10,000 rpm for 10 minutes at 4°C. Supernatants were collected and maintained at -20 °C until required for biochemical assays (alanine and aspartate transferases, alkaline phosphatase activity and oxidative stress indices) within 48 hours.

Estimation of Serum cardiac functional Indices: According to the manufacturer’s instructions (Monobind Inc. USA), the levels of serum creatine kinase, angiotensin converting enzyme and cardiac troponin I (CTPI) was measured using enzyme-linked immunosorbent assay (ELISA) techniques (Acculiteclia microwells). All reagents were reconstituted and brought to room temperature (20–27 °C) as instructed and tests carried out entirely in accordance with instructions.

Estimation of cardiac amino transferases and alkaline phosphatase activity: Alanine and aspartate amino transferase and alkaline phosphatase activities were assayed using a commercial diagnostic Kit Produced by Randox Laboratories Limited, England. The assay protocol adhered strictly to the manufacturer’s instruction.

Estimation of antioxidant and oxidative stress parameters: Oxidative stress indices in the heart were estimated following already existing standard protocols as described by the following authors; Gutteridge and Wilkins (1982) (lipid peroxidation); Misra and Fridovich (1959) (superoxide dismutase (SOD)); Cohen et al. (1972) (catalase (CAT); Elman (1959) (reduced glutathione (GSH)).

Histopathological evaluation of the Heart: Before receiving a transcardial infusion of normal saline and 10% buffered formaldehyde for a histological examination of the heart, the mice in each treatment group from the four groups were given ketamine anaesthesia. The heart was excised after perfusion and put into a 10% formaldehyde solution. It was then left for 24 hours before being moved to a 30% sucrose solution, and it was processed further within 48 hours as previously mentioned (Okhew et al. 2024). An automatic tissue processor was used to treat the excised heart tissue for paraffin wax embedding. This involved dehydrating the tissue through 70%, 90%, and 95% ethanol changes, each lasting 90 minutes. Two xylene changes lasting two hours each were used to achieve clearing, and two paraffin wax changes lasting two hours each were used to infiltrate. Using a rotary microtome, a transverse piece of the heart was cut at 5 μm and Haematoxylin and eosin (H and E) staining technique was done according to Zhang et al. (2017), evaluated, and captured on camera with a light microscope.

Statistical and Data Analysis: Graph pad Prism software, Inc., Lajolla, USA, version 9.5.1 was used to analyse the data using one-way (ANOVA) analysis of variance and the Bonferroni post-hoc test for comparison across experimental groups. At level p<0.05, statistical differences were deemed significant and the Mean ±SEM (standard error of the mean) used for data presentation.

RESULT AND DISCUSSION
The heart is an important organ that controls several significant activities in the body. The heart is primarily concerned with the pumping of blood which aids in the circulation of hormones, nutrients and other vital substances round the body (Suárez et al., 2020). An impact on this organ by exposure to harmful metals like aluminium and cadmium would cause an alteration to these biological processes which could cause deleterious effects that may lead to death. Findings in the current study indicates that there was a significantly reduced activity of creatine kinase in all metal treated groups compared to control however, no significant difference was observed when the metal groups were compared. Creatinine kinase is known as an essential enzyme in the energy coupling system of...
the heart and muscles. Phosphocreatine (PCr) and adenosine diphosphate are produced by the reversible transformation of creatinine and adenosine triphosphate by the enzyme. The enzyme catalyses the reversible conversion of creatinine and adenosine triphosphate to phosphocreatine (PCr) and adenosine diphosphate (Del franco et al., 2022). The observed reduction of CK levels in the groups exposed to the metals indicates an interference of the metals on the functionality of the enzyme through either inhibition of the enzyme or alteration in one of the creatinine metabolic intermediates such as the creatinine transporter (Zervous et al. 2016; Cao et al. 2018). Earlier low serum creatinine kinase has been associated with rheumatoid arthritis an autoimmune and inflammatory condition were the body’s immune set up is programmed to attack healthy cells (Stucki et al. 1996) arising from very weak muscles. This is linkable to a possible high-level weakness of the cardiomyocytes which plays a significant role in the pathological process of heart failure. It also further substantiates the reports of Nascimben et al., (1996) who validated the correlation between a decrease in creatine levels to heart failure and the left ventricular ejection fraction hence the stabilization of creatinine metabolism and signalling functions essentially in heart contraction and imperative in managing heart morbidities. (Balestrino, 2021). Angiotensin converting enzyme is an important enzyme that functions in the regulation of blood pressure by controlling the rate of fluid cum blood flow from the arteries to the body (Danilzyk et al. 2004; Ahmad et al. 2023). A significantly elevated activity of ACE in the Al group may be linked to early onset of sarcoidosis by the prenatal aluminium exposed to the mice. Aluminium and other metals such as silica, barium, cobalt and gold have earlier been implicated in the development of granulomatous lung disease that mimics sarcoidosis (Newman, 1998; Fireman et al. 2016; Bajer et al. 2020; Judson 2020). Though the Cd group had a non-significantly elevated ACE activity, the findings in this study are in contrast to that reported by Brosgéhini-Filho et al., (2015) which noted a reduction in the serum concentration of ACE after acute cadmium exposure but agrees with Júnior et al., (2020) that reported a similar result stating an increase in serum ACE levels in mice exposed to Cd only. The increases signifies that the renin-angiotensin system plays an important role in the toxicity of the cardiovascular system and therefore may also promote the progression of hypertension. Also, its reduction in the coadministration group may signify possible negative response factors from other organs such as the liver and may be significant for the determination of the liver’s health in the co-exposed animals (Lim et al. 2020; Laitsselart et al. 2022; Pan et al. 2023). The significant elevation of CTP1 by cadmium signifies the possible induction of heart attack (myocardial infarction) in the group prenatally exposed to cadmium. This assertion is so because the CTP1 has been identified as a superior marker for the left ventricular malfuncion (Buiten et al. 2015) and that in most cases, wherever there is a rise in CTP, it is indicative of heart damage or a very recent myocardial infarction. Our findings therefore agree with previous studies of Salah and Hafez (2016), which reported increased CTP1 levels in rats exposed to CdCl2 in an oxidative cardiac toxicity model. It also further supports Liu et al., (2021) which observed an elevation of cardiac troponin in mice exposed to cadmium. It is also of great importance to state that in an earlier study from our lab (Ichipi-Ifukor et al. 2019) we had postulated the possible involvement of Cd in the induction of myocardial infarction owing to elevated serum and cardiac AST and alteration in cardiac myofibers. Thus, in line with these conclusions, it can suffice to submit that cadmium causes myocardial damage and leads to the intensification of cardiac injury.

![Fig. 1: Effect of Aluminium and Cd co-exposures on serum cardiac functional markers](image)

**Fig. 1:** Effect of Aluminium and Cd co-exposures on serum cardiac functional markers

**Key:** A: Creatinine Kinase; B: Angiotensin Converting Enzyme; C: Cardiac troponin I (CTP-I)

Bars with varying alphabet superscripts are significantly different (p<0.05).

Fig. 2 shows that there was no significant difference on ALT, AST and ALP activities in the heart of mice prenatally exposed to only Al compared to control and the Al+Cd group. Significant elevation of cardiac ALT, AST and ALP were observed in the group exposed to Cd only compared to control, and Al groups

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while only AST was significantly increased when comparing the Cd and the Al+Cd groups. Findings in this study are similar to those reported by Ichipi-Ifukor et al. (2019). The overall increase in aminotransferase concentrations in the groups exposed to the metals can be attributed to the stress induced on the cells. It has been earlier postulated that in a dysfunctional heart tissue, that there is usually a metabolic shift from fatty acid utilization as major energy pool of the myocardium to the utilization of glucose as the increased hypoxic condition of the heart necessitates an upsurge of oxygen supply for glucose breakdown and utilization (Bertero and Maack, 2018; Nabben et al., 2018; Shi and Qiu, 2022). Based on this therefore, it will suffice to say that the cardiac cells in this study resorted to alternative means of energy sources by channelling amino acids towards energy generation via gluconeogenesis (Cetica et al., 2003; Sanchez and Raja, 2023).

Fig 2: Effect of Al and Cd co-exposures on cardiac amino transferases and alkaline phosphatase

Bars with varying alphabet superscripts are significantly different (p<0.05).

Key: A: Alanine transferase (ALT), B: Aspartate transferase (AST) C: Alkaline phosphatase (ALP)

Fig. 3 shows that there was a significantly reduced levels of lipid peroxidation in the Al group compared to control and the Cd group. The reported significant reduction in the MDA of the Al group is not in consonance with the ability of Al to elevate MDA levels as reported by Hegazi and Elebshany (2019). Likewise, the non-significant change mediated by Cd is contrary to that reported by Ichipi-Ifukor et al. (2022) as Cd mediated rise in cardiac MDA within a 72h period. The reported variation in these studies as compared to the current study may be as a result of the exposure route and duration of exposure. SOD being a second messenger enzyme that functions in the dismutation of super oxides to less harmful products were reported to be non-significant across all treatment groups compared to control. This observation may also be related to dose, route and exposure timelines. It is of great importance to note that similar results have earlier been reported for the testes and liver by our lab (Okhue et al., 2024; Irehievwie et al., 2024) in a related co-exposure regimen and supports in entirety the submission that the handling of the toxic malignancies of Al and Cd co-exposures in the current study did not depend fully on SOD activities. While the reported significant reduction on catalase enzymes and the GSH in the Al, Cd and co-exposure group for GSH only is in tandem to earlier reports implicating Al and Cd in the inhibition of the activities of catalase as well as modification of the GSH function by attaching to its sulfhydryl group along its sulfhydryl homocysteine moiety which further promotes its depletion along the metabolic pool (Asagba et al., 2008; Ameri et al., 2020; Ding et al., 2017; Ichipi-Ifukor et al., 2022).

Fig 3: Effect of Al and Cd co-exposures on cardiac Oxidative stress markers

Bars with varying alphabet superscripts are significantly different (p<0.05).

Key: A: Malondialdehyde (MDA), B: Superoxide dismutase (SOD) C: Catalase (CAT); D: Reduced Glutathione (GSH)

Fig. 4 shows a representative histological feature of the heart of mice exposed to prenatal Al and Cd. The control mice (A) show there was no observable lesion in the heart indicating an intact myofibers and cardiomyocytes and normal heart histology (blue arrows). The group exposed to prenatal Al (B)
displayed intact cardiomyocytes with an early onset of myofiber weakening and necrosis (green arrows). The Cd group (C) and the group exposed to Al+Cd prenatally however shows random disintegration of cardiomyocytes (black arrows), patchy and early onset of myofiber degeneration and necrosis (green arrows) and multiple congestion of blood vessels (white arrows). The features of the heart in these groups are indicative of an abnormal heart histology and development of varying levels of heart disease and malfunction confirming earlier claims in this study of a possible weakness of the cardiomyocytes and pathological heart failure induced by prenatal Cd exposure. The Alterations observed in the histology of the heart in the Cd and Al+Cd groups are similar to those reported in earlier studies relative to Cd toxicity (Kushalf 2009; Arbi et al. 2021; Chou et al. 2023).

Conclusion: Our findings revealed that exposure to aluminium and cadmium during pregnancy altered indices of cardiac function via pathways related to angiotensin, cardiac troponin and oxidative stress signalling which may have impacted directly on the histarchitectural features of the heart. Comparatively, prenatal exposure to Cd alone impacted more negatively to the heart in relation to exposure to either Al only or co-exposure to Al and Cd.

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