Original Research Article

Cardiac Effects of Combined Lead Exposure and Chronic Restraint Stress: Alterations in Biomarkers and Myocardial Health

ABSTRACT

Lead is a toxic metal and an environmental pollutant that has been associated with an increased incidence of cardiovascular diseases while restraint stress is a psychological stress model that can also potentially induce deleterious effects on cardiac functions. The aim of this study is to investigate the cardiac effects of acute lead acetate administration and restraint stress exposure in female Wistar rats. Twenty-four (24) female Wistar rats weighing 180 - 240 grams were randomly divided into four (4) groups (n=6): Control (CTL), Restraint stress alone (RSA), Lead acetate alone (LDA), Lead + Restraint stress (LRS). The duration of the study was 21 days. The LDA group were orally administered 100mg/kg of lead acetate, the RSA group were restrained for 1 hour daily and the LRS were administered lead acetate and restrained for 1 hour daily. Twenty-four hours post last lead administration and restraint exposure, the animals were anesthetized and the electrocardiograph (ECG) of the animals were measured. Thereafter, all animal was sacrificed. Blood was collected via cardiac puncture for biochemical analysis. Results showed altered cardiac conductivity including blood pressure, ECG (heart rate, P-wave, QRS complex) in the animals. Serum creatine kinase (CK), lactate dehydrogenase (LDH) and troponin I (TnI) levels were significantly increased ($p<0.05$) in RSA and LDA groups when compared with control. The LRS group showed a significant increase ($p<0.05$) in CK, LDH and TnI levels when compared with control, lead alone and restraint alone groups. In conclusion, this study showed that exposure to restraint stress and lead has a deleterious effect on cardiovascular function of female Wistar rats.

KEYWORDS: ***Lead acetate, Restraint stress, ECG, Cardiac function markers, Female Wistar rats***

1. INTRODUCTION

Heavy metal poses a major environmental threat to life due to their toxicity even at low level of exposure, longevity and ability to accumulate in the human body via bioaccumulation (Mitra et al., 2022). These metals such as lead, cadmium, cobalt, lead, arsenic and others are characterized by their high atomic mass and density (Wiilliams, 2019). Lead is is a common environmental toxin absorbed into the human body through the respiratory system, gastrointestinal tract and skin (Simal-Gandara, 2022). Lead intoxication has also been associated with an increased incidence of coronary artery disease, stroke, peripheral arterial disease, and an increase in non-high-density lipoprotein cholesterol. However, the mechanism by which lead is implicated in cardiovascular disease is believed to be related to oxidative stress, nitric oxide dysregulation and alteration in the renin-angiotensin-aldosterone system (Hegde et al., 2020).

Stress is a physiological and psychological response to external and internal environmental stimuli (Yang et al., 2024). Stress is a risk factor for the onset of comorbid mental health difficulties and the progression of cardiovascular diseases (Cooke et al., 2020). Restraint stress is one of the most commonly used models to induce psychological stress by placing the animals in a plastic tube in order to restrict their movements (Santha et al., 2016).

The cardiovascular system is a transport system composed of the heart, blood vessels and blood to facilitate the intricate process of blood circulation and nutrients throughout the body (Saghiv*et al.,* 2020). Cardiovascular parameters include systolic blood pressure (SBP), diastolic blood pressure (DBP) and cardiac biomarkers which are enzymes, compounds or proteins which can be quantified in assays. Cardiac biomarkers includes cardiac troponin I, lactate dehydrogenase and creatine kinase (Obeng-Gyasi, 2019; Muralikrishnan et al., 2022).

2.0 Material and Methods

2.1 Chemicals and Compounds

Lead (Kermel, China), Chloroform, Normal saline, distilled water, Formosaline, phosphate buffer saline was purchased from Science laboratory, LAUTECH, Oyo state, Nigeria.

2.2 Study Design

Twenty-four (24) healthy adult female Wistar rats weighing 180-240g were purchased from the Animal laboratory (Oyo state, Nigeria), Department of Physiology and kept under a standardized laboratory environment (12/12 h light/dark cycle). The rats were acclimatized for two weeks and were allowed free access to animal feed and water *ad libitum.* All animals received humane care in compliance with the Guidelines of the Animal Research Ethical committee of Ladoke Akintola University of Technology. This animal experiment was approved by the Institutional Animal Research Ethical Committee (APPROVAL NO: ERCFBMSLAUTECH:059/08/2024).

After acclimatization, the rats were randomly divided into four groups with six (6) rats in each group. Group I represent the control group while groups II, III, IV served as the experimental groups. The table 1 shows animal grouping and summary of experimental procedure:

Table1: Animal grouping and experimental procedures

GROUPS	ADMINISTRATION
Control (CTL)	Rats were given only animal feed and water *ad libitum* for 21 days.
Lead alone (LDA)	Rats were administered lead (100 mg/kg) orally for 21 days.
Restraint stress alone (RSA)	Rats were subjected to restraint stress using wire mesh for 1 hour daily for 21 days.
Restraint stress + Lead (RSL)	Rats were administered lead (100 mg/kg) orally and were subjected to restraint stress using wire mesh for 1 hour daily for 21 days.

2.3 ARTERIAL BLOOD PRESSURE AND ECG Measurement

Twenty-four hours after the last lead administration and restraint stress exposure, the rats were anesthetized with ketamine (50mg/kg) and xylazine (0.75mg/kg) which was administered subcutaneously. Then, the arterial blood pressure (ABP) was measured using tail-cuff method. Gel was applied to the four limbs and chests of the rats and five veterinary

ECG leads was attached to the chest (V) and limbs (RA, LA, RL, LL) each. Each leads were attached to an atraumatic lip electrode, then to the gel pots and the cardiogram was recorded for 60s with the custom-made software accompanying the system.

2.4 Sample preparation

After the ECG measurement, the animals were sacrificed under anesthesia by placing them in desiccator with a chloroform soaked cotton wool. Blood samples was collected via the cardiac puncture into sample bottles. Serum was obtained from the collected blood by centrifuging at 2500 revolutions per minute for 10 minutes. The obtained serum was stored at -80□ until use.

2.5 Biochemical Assays

Cardiac creatine kinase, lactate dehydrogenase and troponin I were assayed using their ELISA kits.

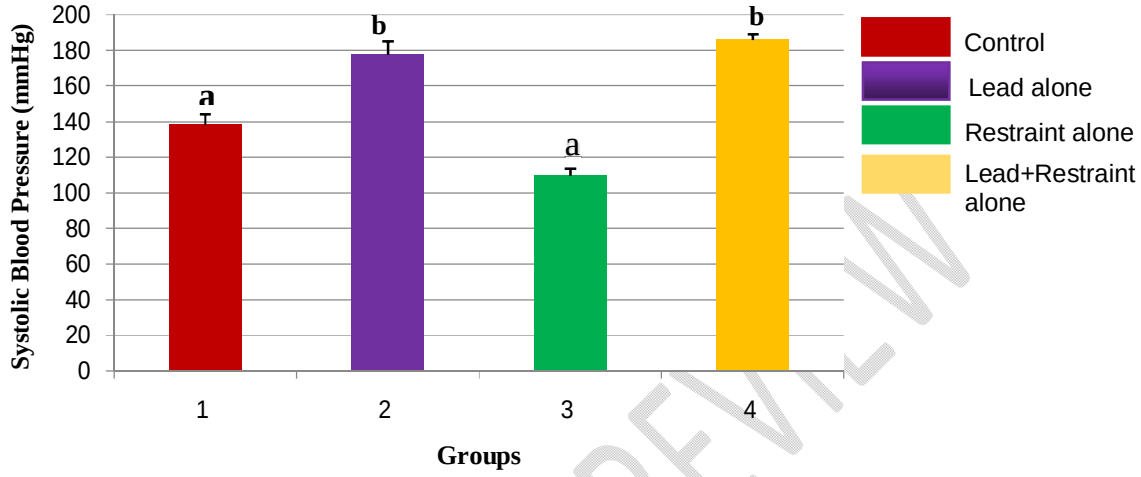
2.6 STASTICAL ANALYSIS

All results obtained are expressed as Mean ± Standard Error of the Mean (S.E.M). Statistical analysis of results were performed using SPSS (version 16). Each mean value was compared by one way analysis of variance (ANOVA) and statistical differences between groups using Duncan's *posthoc*. $P<0.05$ is considered significant.

3. Results and Discussion

Results

A



B

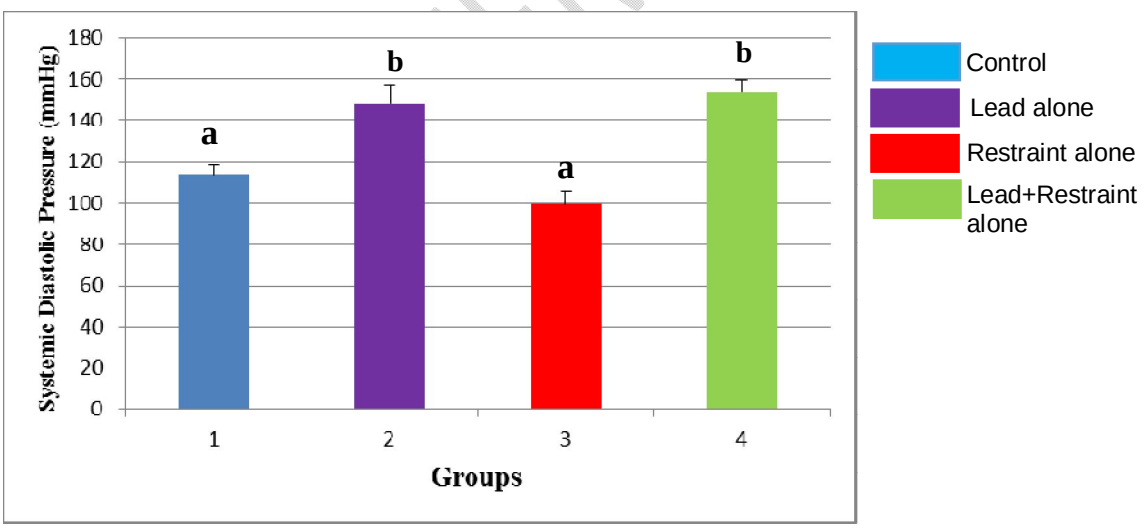


Figure 1 showing the systolic blood pressure (A) and diastolic blood pressure (B) in female Wistar rats.

Values are expressed as mean ± SEM (n=6). Bars with superscripts of different letters are significantly (p<0.05) different from each other. Bars with superscripts of same letters are not significantly (p<0.05) different from each other.

There was a significant increase ($p < 0.05$) in systolic and diastolic blood pressure (mmHg) of lead alone group when compared with control. The restraint stress group showed no statistical difference when compared with control. Lead + Restraint stress group showed a significant increase ($p<0.05$) when compared with control and restraint stress groups but not statistically significant compared to lead alone.

Table 2 The effect of lead actetate administration and restraint stress exposure on Electrocardiogram in female Wistar rats.

Values are expressed as mean ± SEM (n=6). Mean values with superscripts of different letters are significantly ($p<0.05$) different from each other.

Groups	HR	P	PR	QRS	QT	QTC	Ra
CTL	230.00±7.93[a]	19.00±2.89[a]	41.00 ±5.20[a]	14.00±0.58[a]	65.67±2.96[a]	121.67±7.34[a]	0.49±0.02[a]
LDA	287.00±11.93[b]	21.67 ± 1.45[a b]	49.33±4.06[ab]	16.67±0.33[b]	76.00±7.64[ab]	155.00±7.81[b]	0.70±0.09[b]
RSA	237.33±6.49[a]	20.00±2.00[ab]	48.33±1.76[ab]	15.67±0.33[a]	76.33±7.35[ab]	151.33±6.42[c]	0.84±0.13[c]
LRS	270.33±4.33[b]	23.33±0.33[b]	56.33±2.03[b]	17.33±1.08[b]	86.00±7.57[b]	161.00±10.41[d]	0.72±0.06[b]

There was a significant increase ($p<0.05$) in heart rate and Ra of LDA when compared with control. The RSA group showed no statistical significance ($p<0.05$) in HR but Ra was significantly increased ($p<0.05$) when compared to control. LRS group showed a significant increase ($p<0.05$) in HR when compared with control but showed no statistical significance when compared with LDA group. Ra was significantly increased ($p<0.05$) when compared with control but significantly decreased ($p<0.05$) when compared with RSA group. There was no statistical significance in P-wave and PR interval of LDA and RSA groups when compared to control. LRS group showed that P-wave and PR interval was significantly increased compared to control but not statistically significant when compared LDA and RSA. QRS and QTc was significantly ($p<0.05$) increased in LDA and RSA groups when compared with Control. The LRS group showed a significant increase in QRS duration ($p<0.05$) when compared to control but not statistically significant when ($p<0.05$) when compared to LDA and

RSA groups. However, there was a significant increase ($p<0.05$) in QTc of LRS group when compared with control, LDA and RSA groups.

HR: Heart rate, P= Electrical depolarization of the atria, PR= Time from the onset of the P wave to the start of the QRS complex, QRS= Electrical impulse as it spreads through the ventricles and indicates ventricular depolarization, QT= Space between the start of the Q wave and the end of the T wave, QTC= Heart-rate corrected QT interval, Ra= Depolarization of the main mass of the ventricles.

Table 3The effect of lead acetate administration and restraint stress exposure on Pulse rate in female Wistar rats.

Values are expressed as mean ± SEM (n=6). Mean values with superscripts of different letters are significantly ($p<0.05$) different from each other.

GROUPS	VALUE
Control Group	81.00 ± 2.08^{ab}
Lead acetate Group	91.67 ± 7.84^{a}
Restraint stress Group	72.33 ± 1.20^{b}
Lead + Restraint stress group	86.33 ± 7.17^{ab}

There was a significant increase ($p < 0.05$) in pulse rate of lead alone group when compared with control. The restraint stress group showed a significant decrease ($p<0.05$) when compared with control and lead alone groups. Lead + Restraint stress group showed no statistical significance when compared with control. Pulse rate was significantly decreased in the LRS group when compared with lead alone but significantly increased ($p<0.05$) when compared with restraint stress alone group.

Table 4The effect of lead acetate administration and restraint stress exposure on cardiac biomarkers in female Wistar rats.

MARKERS	CTL	LDA	RSA	LRS
Creatine kinase	6.11±1.31[a]	16.44±1.11[b]	12.53±2.11[c]	32.22±2.81[d]
Lactate dehydrogenase	34.28±0.56[a]	69.08±3.33[b]	55.62±1.95[c]	79.88±2.33[d]
Troponin I	6.11±1.31[a]	16.44±1.11[b]	12.53±2.11[c]	32.22±2.81[d]

Values are expressed as mean ± SEM (n=6). Mean values with superscripts of different letters are significantly (p<0.05) different from each other.

There was a significant increase (p<0.05) in Creatine kinase, Lactate dehydrogenase and Troponin I level in lead alone and restraint stress alone group when compared to control. Creatine kinase, Lactate dehydrogenase and Troponin I was significantly increased (p<0.05) in lead + restraint stress group when compared with control, lead alone and restraint stress alone groups.

3. DISCUSSION

Lead acetate is an heavy metal that has been shown to have detrimental effects on cardiovascular system. Restraint stress, however, is a form of psychological stress that can also disrupt optimal cardiovascular health. When these two factors are combined, they can potentially amplify cardiovascular dysfunction. This study focuses on evaluating the effect of lead acetate administration and restraint stress exposure on cardiovascular function in female Wistar rats.

Blood pressure is the lateral pressure exerted by blood flow on the vessel wall per unit area. It comprises of the systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Lin *et al.,* 2024). Result observed in Figure 1 A and B showed that lead acetate group showed a significant increase ($p<0.05$) in the level of systolic blood pressure and diastolic blood pressure compared to control respectively. This study correlates with a previous study where blood lead levels were significantly higher than the control in hypertensive patients (Alghasham*et al.,* 2011). Lead can reduce bioactive nitrogen monoxide (NO) and downregulate soluble guanylate cyclase in vascular tissues (Gambelunghe*et al.,* 2016). It causes a reduction in cyclic GMP production and attenuation of NO activity leading to inhibition of vasorelaxation (Tsoi *et al.,* 2021). Lead has also been proposed to increase angiotensin-converting enzyme activity as well (Simoes *et al.,* 2011). Hence, in this current study, the lead-induced increase in blood pressure can be due to the combination of these molecular effects which increases vascular resistance thereby increasing blood pressure.In the restraint stress group, there was no statistical significance the level of systolic and diastolic blood pressure when compared to the control group. This current study is in contrast to a previous study of do-Vale *et al.,* (2020) where acute restraint stress increases blood pressure in rats but similar to a cohort study where exposure to stress did not alter arterial blood pressure in participants exposed to psychosocial stress (Nwanaji-Enwerem *et al.,* 2022). Restraint stress typically activates the sympathetic nervous system and increasing cathecholamine release, raising blood pressure (Ayada *et al.,* 2015). However, this result indicates that restraint stress did not induce a significant hypertensive response. This may be due to factors like the duration of stress exposure or the intensity of stress sessions. Lead + Restraint stress group showed a significant increase ($p<0.05$) in SBP and DBP when

compared with control and restraint stress groups but not statistically significant compared to lead alone. In this group (LRS), it could be suggested that restraint stress did not exacerbate lead-induced hypertensive effect which suggests that lead toxicity is the dominant factor that altered the blood pressure levels. In the LRS group, one possible explanation to the limitation of restraint stress contribution to high blood pressure can be due to habituation exhibited by the animals to mitigate the effect of restraint stress.

Electrocardiogram (ECG) is one of the vital tests used to record the electrical activity of the heart with cardiac rhythm. It can be used to determine abnormalities associated with cardiac conduction system (Hammad *et al.,* 2018). In Table 2, result showed that the heart rate in lead exposed group was significantly increased ($p<0.05$) when compared with control. Elevated heart rate may be due to the ability of lead (Pb) to alter calcium homeostasis by binding to calcium affinity sites. Lead alters calcium transport system which includes activation of protein kinase C (that activates Ca2+ channel opening) and inhibition of NaK ATPases (which increases calcium influx) ultimately resulting to increased heart rate which can cause hypertension due to increased cardiac output (Ferreira *et al.,* 2017). The restraint stress alone group showed no statistical significance in heart rate of the animals when compared to control. This result correlates with the previous study of Koc and Ekici (2023) where heart rate of rats exposed to acute restraint stress showed no significant difference when compared with the control group. However, a previous study showed that acute restraint stress raised heart rate in mice (Varejkova*et al.,* 2019). Interestingly, this result corroborates the result of SBP and DBP. Result observed in the LRS group showed a significant increase ($p<0.05$) in heart rate when compared with control and restraint stress alone but not significant when compared to lead alone. This result is indicative that lead exposure might have majorly contributed to increase in heart rate with minimal contribution from restraint stress. Another possible explanation to this is that this study might have been conducted over a short period, during which the effect of restraint stress might not have been significant enough to contribute to the lead-induced heart rate increase.

The P-wave is the atrial depolarization while the PR interval represents depolarization of the atrial and the duration of impulse conduction from the atrium to the ventricle (Magnani *et al.,* 2013). Result observed in this study showed a relative increase but no statistical

significance in P-wave and PR interval of lead alone group when compared to control. This result correlates with a previous study where prolonged P-wave and PR-interval was observed in lead-exposed participants (Cheng *et al.,* 1998). Lead exposure can result in first degree atrioventricular block resulting in prolonged PR-interval. Result observed in the RSA group showed no statistical significance in P Wave and PR intervals when compared with control. This result correlates with a previous study where PR interval showed no statistical changes in rats exposed to restraint stress (Nagaraja and Jeganathan, 1999). This type of response for the prolonged stress explains the partial adaptation of the animals to the stressful stimuli. Interestingly, this result is in correlation to the result observed in heart rate. Result observed in LRS group showed that PR interval was significantly increased compared to control and showed a relative increase when compared lead alone and restraint stress alone. This result can be due the lead-induced prolongation of PR interval which can result in atrial-ventricular (AV) block with minimal contribution from restraint stress.

Result observed in the QRS and QTc complex showed of LDA group showed a significant increase when compared with control. This result is in correlation with a nonoccupational cohort study where high QTc and QRS interval was positively related to low level cumulative exposure to lead (Eum *et al.,* 2011). Previous studies generally postulate that prolonged lead may interfere with myocardium function directly via the impairment of calcium channels, cellular signaling pathways, phosphorylation of myofibrils, and Na+/K+-ATPase function which may result to abnormal cardiac conductivity, increasing the risk of sudden cardiac death (Kiełtucki*et al.,* 2017).

QRS and QTc was significantly ($p<0.05$) increased in Restraint stress group when compared with Control. The result observed in this study is similar to a previous study where acute mental stress induced prolonged QTc interval and prolonged QRS duration in male subjects (Bhide *et al.,* 2015). This may be due to autonomically induced repolarization changes. Sympathetic hyperactivation in response to both mental challenge and affective distress increases circulating levels of epinephrine and norepinephrine. The prolonged QT interval is regarded as a marker of imbalanced distribution of sympathetic nervous system activity on the heart; also, QT interval prolongation has been associated with a lowered ventricular fibrillation threshold and with the occurrence of sudden cardiac death (Bhide *et*

*al.,*2015). The prolonged QT and QRS observed in this present study can be as a result of stress-induced autonomical depolarization changes. The restraint stress+lead group showed a significant increase in QRS duration ($p<0.05$) when compared to control but not not statistically significant when ($p<0.05$) when compared to lead alone and restraint stress alone groups. However, there was a significant increase ($p<0.05$) in QTc of LRS group when compared with control, lead alone and restraint stress alone groups. The relative and significant increase observed in this study indicate that the combined exposure to lead and stress can significantly alter the sympathetic nervous system activity on the heart which can ultimately cause sudden cardiac death. However, this study might have been conducted over a short duration for a significant change to be observed in QRS complex between groups.

Pulse rate measures the number of times the arteries constricts and relaxes to create a noticeable pulse (Ismail, 2021). Result observed in Table 3 showed a significant increase ($p<0.05$) in pulse rate of LDA group when compared to control.The result of this study is similar to a previous study where pulse rate was increased in human subjects exposed to chronic low level of lead (Upadhyay *et al.,* 2020). The effect of lead in this study may be as a result of lead-induced sympathetic over-excitation (Geraldes *et al.,* 2016), impairment of the parasympathetic nervous system (Chen *et al.,* 2021) or compensatory tachycardia due to cardiac dysfunction (Skoczynska and Skoczynska, 2012). The restraint stress group showed a significant decrease ($p<0.05$) in pulse rate compared to control. The result of this study is in contrasts to previous studies where exposure to restraint stress increased pulse rate in rats compared to control (Sikora *et al.,* 2016)but also correlates with a numerical study that assessed mental stress using a photoplethysmogram. In this previous study, pulse rate was decreased with increasing heart rate (Charlton *et al.,* 2018). The decreased pulse rate may be due to the duration of the study, frequency of stress sessions or due to increased parasympathetic tone which can result in decreased pulse rate. Pulse rate was significantly decreased in the LRS group when compared with lead alone but significantly increased ($p<0.05$) when compared with restraint stress alone.

Creatine kinase is an intracellular enzyme that catalyzes the reversible phosphorylation of creatine by adenosine triphosphate. It is a central regulator of cellular energy and a specific biomaker for myocardial muscle damage (Moghadam-Kia *et al.,* 2016;

Aujla *et al.,* 2024). In addition, lactate dehydrogenase is a cytoplasmic enzyme that catalyzes the conversion of pyruvate to lactate at the end of glycolysis. It is a marker of cell and tissue damage (Geng *et al.,* 2023). Result observed in Table 4 showed a significant increase ($p<0.05$) in serum CK and LDH in the LDA group when compared to control. This result correlates with previous study of Elgharabawy*et al.,* (2021) where serum CK and LDH was increased in rats exposed to lead resulting to cardiotoxic effects. Lead can inhibit gap junction intercellular communication (GJIC) which may promote cell apoptosis leading to cadiomyocytes damage leading to the leakage of creatine kinase (Wang *et al.,* 2023). High ATP requirements of cardiac tissue are achieved mostly by aerobic metabolism through oxidative phosphorylation. It has been reported that lead intoxication provokes damage in the mitochondria membrane, which may lead to increase in lactate dehydrogenase activity and decreased ATP synthesis (Wang et al., 2011). Hence, increased activity of LDH in lead-exposed rats suggest a compensatory glycolytic response of the myocardium to counteract the mitochondrial dysfunction and ATP depletion induced by lead exposure.

Serum CK and LDH was significantly increased ($p<0.05$) in the restraint alone group when compared with control. Results observed in the restraint alone group is in correlation with previous studies where restraint stress induced accumulation CK-MB and LDH in animals (Pergolizzi *et al.,* 2017). Stress-induced tissue hypoxia can result to production of reactive oxygen species in the mitochondria increasing the rate of lipid peroxidation and oxidation of creatine kinase. This predetermine the disruption of energy transport from the mitochondria to the cardiomyocytes ultimately leading to myocardial damage (Shvets *et al.,* 2023). Furthermore, chronic stress can induce epinephrine release which enhances LDHA-dependent metabolic activity which increases the activity of lactate to enhance glycolysis and decreases cellular ATP levels ultimately resulting in myocardial damage (Cui *et al.,* 2019). Increased serum creatine kinase and lactate dehydrogenase activity in this study suggests stress-induced myocardial damage. Serum CK and LDH levels was significantly increased ($p<0.05$) in the LRS group when compared to control, lead alone and restraint stress alone groups. The current result might have resulted from the combined mechanisms of lead-induced disruption in ATP synthesis, oxidative damage and energy transport from the mitochondrial disruption caused by restraint stress.

Cardiac troponin I (cTnI) is one of the highly sensitive and specific biomaker of cardiac damage (Afsar *et al.,* 2019). Result observed in Table 4 showed cardiac troponin I was significantly increased ($p<0.05$) in lead alone group when compared with control. This result correlates with a previous study where cTnI levels was increased in the heart of lead-intoxicated mice (Baghshani*et al.,* 2020). Intracellular calcium is particularly important in cardiac regulation (Zhu *et al.,* 2024). Lead acetate can interfere with calcium signaling in the myocardium through endoplasmic reticulum stress (Zhang *et al.,* 2022). Hence, in this current study, increased cTnI levels might have resulted from the disruption of the endoplasmic reticulum which can induce cell death resulting in the leakage of cTnI into the bloodstream. In this present study, cTnI was significantly increased in restraint stress alone when compared with control. This result is similar with a previous study where chronic restraint stress increased serum cardiac troponin T in female rats (Cairns *et al.,* 2024). Daily exposure to psychological stress can trigger myocardial ischemia which can increases cell wall permeability due to recurrent wall injury leading to release of cytosolic troponin (Hammadah*et al.,* 2018). Serum cTnI levels was significantly increased in the LRS group when compared with control, lead alone and restraint stress alone groups. The current result might have resulted from the combined mechanism of lead-induced endoplasmic reticulum stress and stress-induced ischemia.

4. CONCLUSION

In conclusion, this present study has shown that the combined exposure to lead and chronic stressors caused alteration in cardiac functions resulting to myocardial damage.

Institutional Review Board Statement

This study was conducted following the guidelines set by the Animal Ethical Committee of the Faculty of Medical sciences, Ladoke Akintola University of Technology, Oyo, Nigeria and the regulations were adhered to throughout the research process.

Informed Consent Statement

Not applicable.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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