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Metabolic Effect of Acute Lead and Restraint Stress Exposure on Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lead is a common environmental toxicant while restraint stress is primarily a psychological stressor. Lead exposure and psychological stressors, are known to exert adverse effects on metabolic health independently. The aim of this study is to investigate the metabolic effects of acute lead 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),

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Restraint stress alone (RSA), Lead acetate alone (LDA), Restraint stress + Lead acetate (RSL). The duration of the study was 21 days. The lead acetate alone group were orally administered 100mg/kg of lead acetate, the restraint stress alone group were restrained for 1 hour daily and the restraint stress + lead group were administered lead acetate and restrained for 1 hour daily. Twenty-four hours post last lead administration and restraint stress exposure, all animals were anesthetized and sacrificed. Blood was collected via cardiac puncture for biochemical analysis. Results showed serum low-density lipoprotein (LDL), triglycerides (TAG) and total cholesterol (CHO) levels in lead alone and restraint stress alone groups were significantly increased ($p<0.05$) compared with control. The restraint + lead group showed a significant increase in serum LDL, TAG and CHO levels when compared with control, lead alone and restraint stress alone groups. Serum HDL levels showed no significant difference across all groups. Serum glucose, insulin and cortisol levels in lead alone and restraint stress alone groups were significantly increased ($p<0.05$) when compared with control. The restraint + lead group showed a significant increase ($p<0.05$) in serum glucose, insulin and cortisol 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 significant effect on the metabolic profile of female Wistar rats.

Keywords: Lead acetate; restraint stress; biochemical parameters; female Wistar rats.

1. INTRODUCTION

Heavy metals are a group of naturally occurring metallic elements characterized by their high density and potential toxicity at even low concentrations [1]. These elements including lead, mercury, cadmium, arsenic, and others, can be found in the Earth's crust but are also released into the environment through human activities, such as industrial processes and pollution [2]. Lead acetate, also known as lead diacetate, is a toxic heavy metal that accumulates in the human body and can cause severe health problems such as increased risk of high blood pressure, cardiovascular disease, and kidney damage. Additionally, studies have linked lead exposure to impaired glucose homeostasis, insulin resistance, visceral adiposity and increase endoplasmic reticulum stress [3].

Stress is a complex physiological and psychological response to external and internal environmental stimuli [4]. Restraint stress is a widely used experimental paradigm in behavioural neuropsychatric disorders [5]. This method involves the immobilization of animals, typically rodents in a confined space, which induces both physical and psychological stress responses [6]. Research has shown that such stressors can lead to significant changes in behavior, neuroendocrine function, and brain morphology, making them valuable for studying the mechanisms underlying stress-related conditions.

Metabolic profile is a comprehensive analysis of an organism's metabolic state, which involves measuring a wide range of endogenous and exogenous molecules [7]. Metabolic profile includes insulin resistance, pancreatic beta cell function, glycated hemoglobin and lipid profile [8]. Exposure to stress, both acute and chronic stress, are involved in metabolic dysfunction [9]. However, studies involving the combined influence of restraint stress and lead acetate on metabolic profile of female Wistar rats is limited. Therefore, this study seeks to evaluate the effect of restraint stress and lead acetate exposure on the metabolic profile of female Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Compounds

Lead acetate (Kermel, China), Chloroform, Normal saline, distilled water, Formosaline, phosphate buffer saline were 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 Department of Physiology, Animal Laboratory (Oyo state, Nigeria) 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.*

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 group designate are: I = Control group (CTL), II = Restraint Stress Alone (RSA), II I= Lead Alone (LDA) and IV = Restraint Stress + Lead (RSL). The table below shows animal grouping and summary of experimental procedure:

Table 1. Animal grouping and experimental procedures

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

2.3 Sample Preparation

Twenty-four hours after the last lead acetate administration and restraint stress exposure, the animals were anesthetized 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 for 10 minutes. The obtained serum was stored at -80℃ until use.

2.4 Biochemical Assays

High-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol (CHO) and triglycerides (TG) were assayed using commercial kits and standardized methods. Serum Insulin and Cortisol were assayed using ELISA kits. Serum glucose was assayed using a glucometer.

2.5 Stastical Analysis

All results obtained are expressed as Mean ± Standard Error of the Mean (S.E.M). Statistical analysis of the glucose, insulin and cortisol results were performed using GraphPad Prism (version 5.0). Each mean value was compared by one way analysis of variance (ANOVA) and statistical differences between groups using Tukey's *posthoc* test. Lipid profile results were performed using SPSS (version 16.0) with Duncan *posthoc*. $P<0.05$ is considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

Serum CHO, LDL and TAG levels were significantly increased ($p<0.05$) when compared with control. The restraint + lead group showed a significant increase in serum CHO, LDL and TAG levels when compared with control, lead alone and restraint stress alone groups. Serum HDL levels showed no significant difference across all groups.

Effect of Restraint stress and Lead acetate administration on serum glucose (A), insulin (B) and cortisol (C) in female Wistar rats. Serum glucose, insulin and cortisol levels in lead alone and restraint stress alone groups were significantly increased ($p<0.05$) when compared with control. The restraint + lead group showed a significant increase ($p<0.05$) in serum glucose, insulin and cortisol levels when compared with control, lead alone and restraint alone groups.

3.2 Discussion

Result observed in the lead alone group is consistent with previous research of Alya et al., [10] where cholesterol (CHO), low-density lipoprotein (LDL) and triglycerides (TAG) concentration were significantly increased following lead intoxication in all treated groups compared to their relative controls. Lead accelerates lipid peroxidation and degradation of polyunsaturated membrane lipids and lipoproteins resulting in alteration of lipid metabolism [11]. Increased cholesterol levels may be due to lead-induced activation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and HMG CoA synthase [12] or due to inhibition of cytochrome P-450 resulting in the limitation of bile biosynthesis which is the only significant route for the elimination of cholesterol [10]. Result observed in the restraint stress group compared to control is consistent with the previous study of Ahn et al. [13] where restraint stress induction increases LDL-cholesterol, triglycerides and total cholesterol levels by 150-170%. The result of this current study showed that CHO, LDL and TAG levels

was significantly increased in restraint + lead groups. This result indicates that the combination of restraint stress and lead greatly induces alteration of lipid metabolism.

Figure Showing the effect of restraint stress and lead acetate administration on metabolic parameters in female Wistar rats.

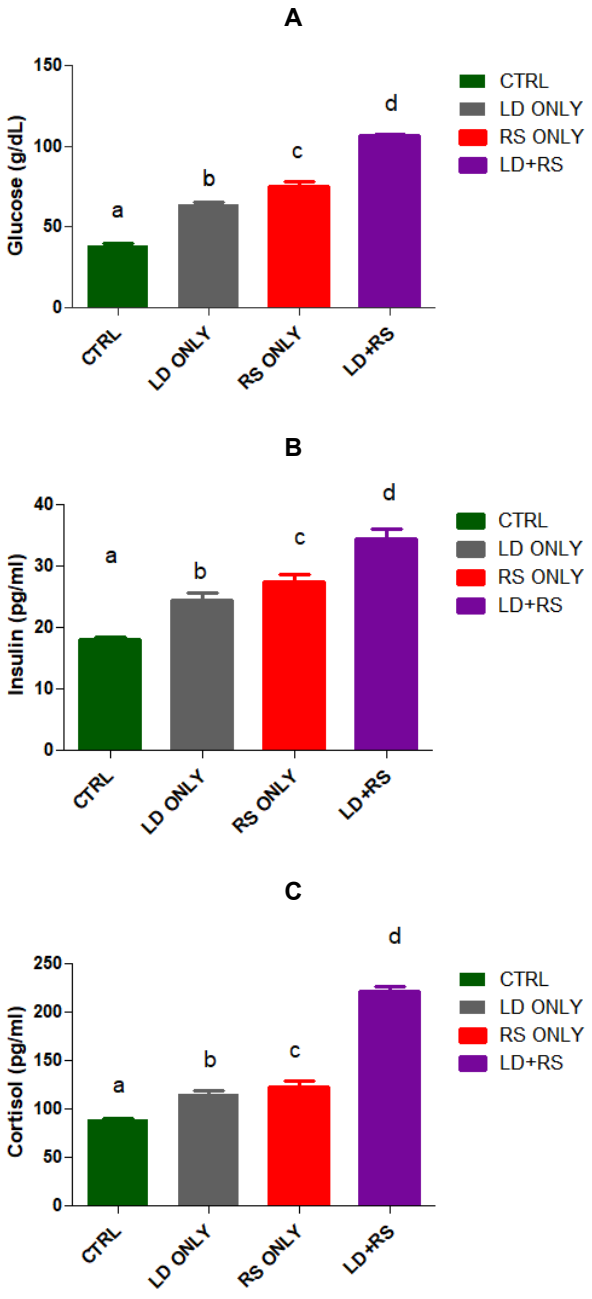

Fig. 1. Effect of restraint stress and lead acetate administration on metabolic parameters in female Wistar rats

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.

Table 2. Effect of restraint stress and lead acetate administration on lipid profile in female Wistar rats

	CTL	RSA	LDA	RSL
CHO (mg/dl)	108.77±5.22[a]	170.12±4.47[b]	167.85±4.45[b]	189.12±4.56[c]
LDL (mg/dl)	42.33±2.42[a]	60.42±2.56[b]	84.68±3.37[c]	90.56±5.22[d]
HDL (mg/dl)	35.52±2.33[a]	38.18±3.62[a]	31.82±2.2[a]	39.22±2.56[a]
TAG (mg/dl)	56.01±2.56[a]	75.11±3.65[b]	90.65±4.41[c]	91.56±4.56[c]

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

High density lipoprotein (HDL) levels showed no statistical significance across all groups. HDL levels are tightly regulated by the body to maintain lipid homeostasis. Despite external stressors or exposures, the body may prioritize maintaining HDL levels within a certain range [14]. This study might have been conducted over a relatively short period, during which changes in HDL levels might not have been significant enough to detect differences between groups. Other mechanisms or compensatory responses within the body might be at play thus, mitigating the effects of lead exposure or stress on HDL levels.

Serum glucose, insulin and cortisol were significantly increased in the restraint stress alone and lead alone groups compared with their relative controls. Results observed in the restraint stress alone group is in correlation with previous studies where fasting serum glucose concentration, insulin and cortisol concentration were elevated in restraint group when compared with control indicating that restraint stress exacerbated glucose intolerance and insulin resistance in rats [15,16]. Increased glucose levels may be related to the enhanced activity of the hypothalamic-pituitary adrenal axis during stress resulting in increased secretion of adrenocorticotropic hormone and corticosteroids into the circulation. The release of ACTH increases cathecolamine production from the adrenal to mobilize carbohydrate reserves from the tissues resulting to elevated blood glucose levels [16]. Insulin resistance can be due to stress-induced corticosteroid secretion. This is consistent with similar reports in which insulin resistance can be induced by cortisol administration in rodents [17].

Results observed in the lead alone group correlates with previous studies in which both fasting glucose and insulin levels was impaired in rats exposed to lead. Impairments of glucose tolerance may be as a result of defects in insulin secretion and/or insulin sensitivity. In correlation to previous studies, lead was significantly associated with higher ACTH:CORT ratios, which is consistent with an decreased adrenal response to endogenous ACTH. Exposure to lead can impair negative feedback loop of the hypothalamic-pituitary adrenal axis leading to higher adrenocorticotropin release during stress response [18]. Serum glucose, insulin and cortisol levels was significantly increased in restraint + lead groups compared to control, restraint alone and lead alone groups. The current result might have resulted from the combined mechanisms of lead-induced alteration of the hypothalamic-pituitary adrenal axis, glucose intolerance and insulin insensitivity caused by restraint stress resulting in metabolic dysfunction [19].

4. CONCLUSION

In conclusion, this present study have shown that the combined exposure to lead and chronic stressors exacerbated the dysregulation of lipid metabolism and glucose homeostasis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

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).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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