Original Research Article

Effect of cadmium chloride administration and restraint stress on kidney antioxidants in female Wistar rats.

ABSTRACT

Heavy metal such as cadmium is toxic and the kidney is one of its major target organs while restraint stress is a model of chronic stress used to mimic human psychological stress. This study aimed to evaluate the effects of restraint stress and cadmium administration on kidney antioxidants in female Wistar rats. 24 female Wistar rats weighing 180-220g were randomly divided into 4 groups (n=6): Control (CTL), Restraint stress alone (RSS), Cadmium alone (CCC), Cadmium + Restraint stress (RSC). Body weights of all the rats were monitored. The experimental groups were subjected to cadmium chloride 100 mg/kg b.w.orally and restraint stress for 30 minutes daily using wire mesh for 21 days. Twenty-four hours after the last cadmium chloride and restraint stress exposure, all rats were anesthetized and sacrificed. The kidneys was excised, homogenized and analyzed for antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), lipid peroxidation (Malondialdehyde). Result obtained showed a significant ($p<0.05$) decrease in the renal Superoxide dismutase, body weight in Restraint stress alone and Cadmium alone groups when compared to Control. There was a significant ($p<0.05$) decrease in the renal catalase in Cadmium group when compared to control. In renal glutathione peroxidase, there was a significant ($p<0.05$) decrease in Restraint stress alone and Cadmium alone groups when compared to Control. Furthermore, there was a significant ($p<0.05$) increase in malondialdehyde in Restraint stress alone and Cadmium alone groups when compared to Control. Also, malondialdehyde showed a significant ($p<0.05$) increase in Cadmium + Restraint stress group when compared to both Restraint stress alone and Cadmium alone groups. Nitric oxide was significantly ($p<0.05$) decreased in Cadmium alone group when compared to Control. This study concludes that kidney exposure to cadmium and

Comment [NM1]: Heavy metals, such as cadmium, are toxic, and the kidney is one of their major target organs. Restraint stress, on the other hand, is a model of chronic stress used to mimic human psychological stress

Comment [NM2]: How was it chosen?

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restraint stress decreased body weight and antioxidant defense capacity, increased lipid peroxidation which may result into adverse renal effects.
Keywords: Cadmium chloride, restraint stress, antioxidant enzymes, lipid peroxidation, Kidney.

INTRODUCTION

The kidneys are important organs responsible for maintaining the body's water balance, acid-base equilibrium, and electrolyte levels by regulating filtration and absorption processes (Imenez Silva et al., 2022).The kidney is highly susceptible to any form of toxicity and one of the major public health issue is kidney disease caused by environmental toxins (Xu et al., 2018). It is documented that with prolonged cadmium exposure, roughly 50 % of the absorbed cadmium accumulate in the kidneys leading to a decrease in kidney antioxidant system (Bautista et al., 2024). The proximal tubules of the nephron are the major target of cadmium toxicity (Fauzi et al., 2023). Furthermore, continuous exposure to cadmium can induce glomerular damage resulting in a decline in glomerular filtration rate (GFR), proteinuria, polyuria and albuminuria which can eventually result in renal failure (Prozialeck et al., 2012; Koushki et al., 2024).

Cadmium (Cd) is a toxic heavy metal that contributes greatly to environmental pollution (Hayat *et al*., 2019). Among the various environmental pollutants, cadmium is ranked as the seventh most hazardous substance indicating the severity of cadmium exposure (Andjelkovic et al., 2019). Cadmium is a naturally occurring heavy metal found in the earth's crust, other sources includes industrial activities in which it is produced as a byproduct of mining, electroplating, cement production and pollution while the non-industrial sources are through smoking, contaminated food and water (Jagaba et al., 2024).There are various route to exposure in which cadmium can gain entry into the human body which includes the respiratory system, gastrointestinal tract and skin (Ebrahimi et al., 2020). Exposure to cadmium poses a severe health related problems to different body systems including the reproductive, renal, hematological, hepatic and nervous system (EL-Hengary et al., 2023).

Stress is a multifaceted physiological and psychological response triggered by either internal or external stressor (Ovsiannikova et al., 2024).Restraint stress is model widely used to induce stress related

symptoms in animals (Van *et al*., 2022). In a stressful situation such as repetitive stress, there is increased metabolism resulting in generation of free radicals (Srivastava and Kumar, 2015). However, the continuous production of free radicals leads to an imbalance between the antioxidant and oxidant system resulting in oxidative stress which can eventually lead to kidney damage (Daenen et al., 2019). Research has shown that exposure to acute stress reaction is associated with risks of acute and chronic kidney disease (Su et al., 2021).

This study therefore sought to evaluate the combined effects of restraint stress and cadmium chloride administration on kidney antioxidants of female Wistar rats.

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2. MATERIALS AND METHODS

2.1 Reagents

Cadmium chloride ($CdCl_2$)(Kermel, China), Normal Saline, Chloroform, Buffered formalin, distilled water was purchased from Department of Pure and Applied Chemistry, LAUTECH, Oyo state, Nigeria

2.2 Experimental Planning and Animals

The experimental rats were kept in a typical laboratory environment (12/12h light/dark cycle). The rats were acclimatized for two weeks with access to feed and water *ad libitum*. All protocols and treatment procedures were done according to the Institutional Animal Care and Use Committee (IACUC) guidelines, in strict compliance with the National Institutes of Health (NIH) guideline for the care and use of laboratory animals.

The duration of the experiment is 21 days. The animal groups and their treatments are:

GROUP 1: Control (CTL). Animals in control group were given only feed and water throughout the course of the experiment.

GROUP 2: Restraint stress (RSS). Animals were subjected to restraint stress using wire mesh for 30 minutes daily for 21 days.

GROUP 3: Cadmium chloride (CCC). Animals were orally administered with 100mg/kg/b.w. of cadmium chloride daily for 21 days.

GROUP 4: Cadmium chloride + Restraint stress (RSC). The rats were orally administered with 100mg/kg/b.w. of cadmium chloride daily for 21 days and were at the same time subjected to restraint stress using wire mesh for 30 minutes daily for 21 days.

Animals subjected to restraint stress were placed in a prone position inside a wire mesh. It was ensured that the head and neck were not compressed to prevent pain induction in the rats.

2.3 Collection and Processing of Samples

Twenty-four hours after the last oral administration of cadmium chloride and restraint stress, the rats were each placed in a desiccator containing chloroform-soaked cotton wool to anesthetize them. The kidney tissues from each rat were then excised, weighed and homogenized in ice-cold Phosphate-buffer saline after being rinsed in ice-cold saline buffer (20mM Tris-HCl, 0.14M NaCl buffer, pH 7.4). The homogenate was then centrifuged in order to examine the oxidative stress markers in the kidney.

2.4 Biochemical Tests

2.4.1 Evaluation of Renal Antioxidant Parameters

Commercial kits purchased from Bio-diagnostic (Cairo, Egypt) were used to evaluate the activities of glutathione peroxidase (GPx), catalase (CAT), malondialdehyde (MDA) and superoxide dismutase (SOD) in the kidney tissues in accordance to the attached enclosed pamphlets.

2.5 Analysis of Statistics

SPSS (version 16.0) was used for all statistical analyses. All results obtained are expressed as Mean ± Standard Error of the Mean (SEM). Data were analyzed using one-way ANOVA and Duncan's *posthoc* test for multiple comparisons. P value < 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1: Effect of restraint stress and cadmium chloride administration on the body weight gain in female Wistar rats.

Body weight gain (grams)	CTL	RSS	CCC	RSC
	50.60 ± 2.14^{a}	21.30 ± 1.53^{b}	5.60 ± 0.69^{c}	0.50 ± 0.04^{d}

Body weight gain= Final body weight- Initial body weight (grams). Values are expressed as mean ±SEM (n= 6). Mean values with superscript of different letters are significantly ($p<0.05$) different from each other. Mean values with superscript of same letters are not significantly different from each other.

There was a significant ($p<0.05$) decrease in body weight in RSS and CCC groups when compared to CTL. Also there was a significant decrease ($p<0.05$) in RSC group when compared to both RSS and CCC groups.

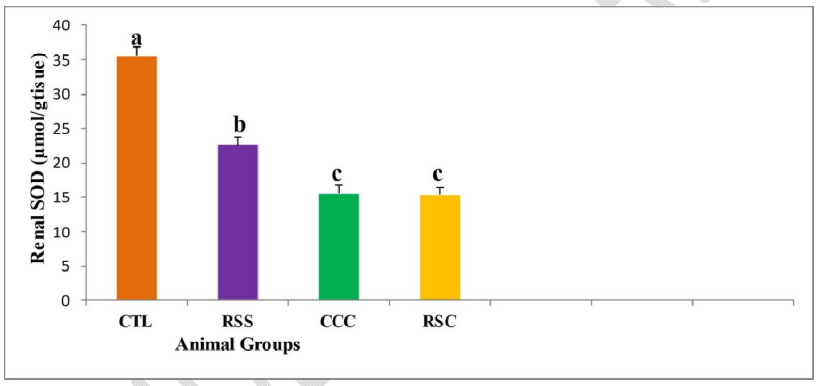

Figure 1: Effect of Cadmium chloride administration and restraint stress on renal superoxide dismutase in female Wistar rats.

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

There was a significant ($p<0.05$) decrease in the renal Superoxide dismutase in RSS and CCC groups when compared to CTL. Renal SOD was significantly ($p<0.05$) decreased in RSC group when compared with RSS group but not statistically significant when compared to CCC group.

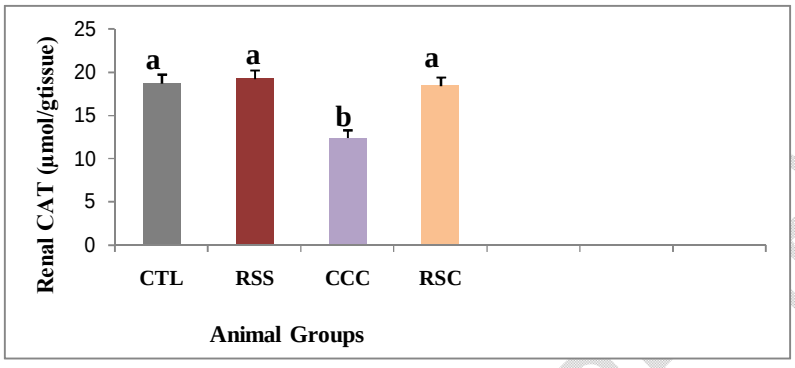

Figure 2: Effect of Cadmium chloride administration and restraint stress on renal catalase in female Wistar rats.

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

There was no significant difference in renal catalase level of RSS group but was significantly ($p<0.05$) decreased in CCC groups when compared to control. There was no significant difference renal CAT levels in the RSC when compared to RSS group but was significantly ($p<0.05$) increased when compared to CCC groups.

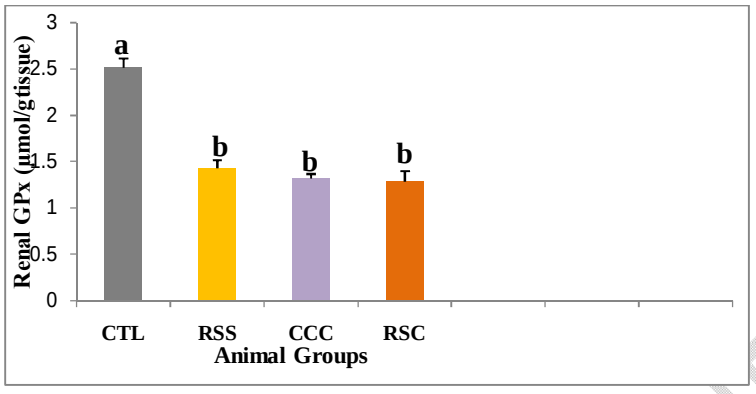

Figure 3: Effect of Cadmium chloride administration and restraint stress on renal glutathione peroxidase in female Wistar rats.

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

There was a significant ($p<0.05$) decrease in the renal glutathione peroxidase in RSS and CCC groups when compared to CTL. However, there was no significant difference in the RSC group when compared to the RSS and CCC groups.

Table 2: Effect of restraint stress and cadmium chloride administration on the renal malondialdehyde in female Wistar rats.

Renal MDA (nmol/gtissue)	CTL	RSS	CCC	RSC
	18.27±1.18[a]	25.72±1.56[b]	35.38±2.11[c]	41.34±1.57[d]

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

There was a significant ($p<0.05$) increase in malondialdehyde in RSS and CCC groups when compared to CTL. Also there was a significant increase ($p<0.05$) in RSC group when compared to both RSS and CCC groups.

Table 3: Effect of restraint stress and cadmium chloride administration on the renal nitric oxide in female Wistar rats.

Renal NO (nmol/gtissue)	CTL	RSS	CCC	RSC
	1.20 ± 0.02^{a}	1.19 ± 0.06^{a}	0.56 ± 0.06^{b}	0.55 ± 0.07^{b}

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

There is no significant difference in renal NO levels in the RSS group when compared to the CTL group. There was a significant ($p<0.05$) decrease in nitric oxide in CCC group when compared to when compared to CTL. Furthermore, there was a significant ($p<0.05$) in renal NO levels of RSC group when compared to RSS group but not statistically significant when compared to CCC groups.

4. DISCUSSION

There is continuous exposure to cadmium in the modern world due to increase pollution in the environment (Genchi et al., 2020). This poses risk to the overall health of individual exposed to this environmental pollutant. The kidney is identified as one of the major target organ to cadmium-induced

toxicity in which oxidative stress is a key mechanism in cadmium-induced toxicity (Yang and shu, 2015).Also, psychological stress is on the rise in the society as a result of socioeconomic instability, professional pressures and various environmental influences which increase the risk of different diseases (Kraft and Kraft, 2021). This present study evaluated the effect of cadmium chloride exposure and restraint stress on the kidney antioxidant.

Body weight is one of the major indicators of toxin-induced effects (Liu et al., 2019). In this study, exposure to restraint stress produceddecreased body weight in female rats. This is in contrast to previous studies where reduction in body weight gain triggered by subchronic restraint was lesser in female than in male rats (Viera et al., 2018). Olave et al., (2022) also observed that females decreased their food consumption with no variations in body weight but males decreased their body weight gain. Proopiomelanocortin (POMC) neurons are situated in the hypothalamic arcuate nucleus where they produce anorexigenic aMSH (alpha-melanocytes-stimulating hormone) to regulate both food intake and energy homeostasis (Song and Choi, 2023). Exposure to repetitive stress can result to hyperactivation of the Pro-opiomelanocortin neurons which can result to decrease in feeding behaviour resulting to decrease in body weight. Decreased body weight gain was also observed in the cadmium chloride group as observed in Table 1. In some studies conducted with mice and rats, oral administration of cadmium did not affect body weight (Duranova et al., 2014; He et al., 2020). However, a study conducted by Haeri et al. (2022), cadmium which was added to the drinking water of mice, reduced the appetite and weight of mice. Decreased body weight in cadmium-exposed group is suggestive of reduced appetite

According to this study, in fig 1, there was a significant ($p<0.05$) decrease in the renal SOD and GPx (figure 3) with an increase in MDA levels (Table 2) in the RSS and CCC groups when compared to control. The relationship between stress and cellular damage caused by free radicals has been well established in previous studies. Pal et al. (2023) conducted a study utilizing Wistar rats to investigate the effects of acute restraint stress on markers of oxidative stress. The experiment findings showed that stress reduced SOD levels with an iincreased activity of MDA. Another study conducted by Samarghandian et al., 2017 showed that restraint stress induced for 1 hr every day for 21 consecutive days decreased GPx in comparison to the normal rats. These findings correlates with the result observed in this study. Superoxide dismutase (SOD) is an enzyme that is highly senstive to oxidative stress. It

catalyzes the dismutation of superoxide ions into oxygen and hydrogen peroxide (Jomova et al., 2024), while GPx is one of the key enzymes vital in regulating glutathione homeostasis and sustaining cellular integrity in tissues. Exposure to stress has been linked to excessive reactive oxygen species production, leading to impaired kidney integrity and cellular membrane function (Nwogueze et al., 2023). Decrease in SOD and GPx levels across all groups observed in this study was due to oxidative stress, which is a disturbance in the balance between antioxidant defense and the production of free radicals. Overproduction of free radicals can also lead to lipid peroxidation which can change membrane integrity and then lead to tissue damage (Samarghadian et al., 2016). The mechanism of stress-induced MDA in kidney tissues involves the interactions between cell proteins and lipids resulting in the generation and release of free radicals, and consequently cellular damage, which at an extreme level interferes with the structural and functional integrity of cells and their respective organelles' membrane.

Cadmium could directly alter cellular enzymes by inducing the generation of reactive oxygen species followed by development of oxidative stress in the target organ (Ogunrinsola et al., 2016). Moreover, cadmium stimulates lipid peroxidation-induced tissue damage. In a previous study, cadmium exposure decreased SOD and GPx levels while increasing lipid peroxidation (MDA) values in experimental rats in comparison to the control (ref). This finding is in correlation to our present study where SOD and GPx were found to be significantly decreased and increase in lipid peroxidation in Wistar rats.This observation implies that Cd intoxication induced oxidative stress via the production of superoxide ions, H_2O_2, hydroxyl radicals and nitric oxide at the tissue level (Genchi et al., 2020; Poli et al., 2022). Result observed in the RSC group suggests that the combined exposure to restraint stress and cadmium greatly altered antioxidant enzymes activity and promotes overproduction of free radicals leading to cellular damage and interferes with kidney tissue integrity.

Catalase is an antioxidant enzyme made up of four iron atoms that catalyzes the conversion of hydrogen peroxide and lipid peroxides into water and oxygen (Anjum et al., 2016). Previous studies have shown decreased CAT levels (Pal et al., 2020) and increased CAT levels (Abdulrauf et al., 2018) in rats exposed to restraint stress. However, in fig 2, there was no signifcance in CAT level of restraint stressed-rats when compared to control. This could imply that the duration of restraint stress exposure may have not been

long enough to provide any significant change in CAT activity. The cadmium-exposed group showed significant ($p<0.05$) decrease in renal CAT levels when compared to control. This might be due to cadmium competing with iron to bind to catalase or disrupting iron absorption which increased the generation of reactive oxygen species (ROS) resulting in oxidative stress and renal damage (Liu et al., 2019).The RSC group showed no significance in CAT level when compared to control and CCC group which is suggestive that the duration may have not been long enough to provide any significant change in CAT activity.

Nitric Oxide (NO) is a free radical that plays important roles in regulation of renal haemodynamics, and long-term control of blood pressure (do Vale et al., 2023). A previous study have demonstrated decreased NO production in borderline hypertensive rats exposed to crowding stress (Bernatova et al., 2018). Another study observed a prolonged period of repeated restraint, 7 and 14 days markedly reduced neuronal NOS in the hippocampus (Gadek-Michalska et al., 2015). In fig 2, there was no signifcance in NO level of restraint stressed-rats when compared to control. Long-term stress may lead to alterations in NO production. Hence, the current study implies that the duration of restraint stress exposure may have not been prolonged enough to provide any significant change in NO production.

Since the kidneys are major target organs of Cd toxicity, indirect cardiovascular effects could arise secondarily to renal injury through damage to both the vascular endothelium and vascular smooth muscle cells (Modlinger et al., 2004). In a previous study by Kukongviriyapan et al. (2014), a significant increase in blood pressure was observed in association with decreased availability of NO in cadmium-induced mice. The present study has demonstrated a significantdecrease in NO level observed in the cadmium alone group (Table 3). Decreased NO availability is suggestive that large amount of O2- rapidly reacted with NO to form peroxynitrite (ONOO-), which is a potent free radical that switch eNOS via oxidation of tetrahydrobiopterin, from a NO-getering to a superoxide-generating enzyme (Matovic et al., 2015). The result observed in the combined cadmium and restraint stress group suggest that cadmium majorly contributed to decreased NO availability.

5. CONCLUSION

In conclusion, our findings revealed that kidney exposure to the combined effect cadmium and restraint stress led to a significant decrease in body weight, antioxidant defense capacity, intensifies free radical production and ultimately resulted in nephrotoxic damage.

DISCLAMIER (ARTIFICAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as large language models (Chatgpt, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

This study was conducted following the Institutional Animal Care and Use Committee (IACUC) guidelines, in strict compliance with the National Institutes of Health (NIH) guideline for the care and use of laboratory animals.

INFORMED CONSENT STATEMENT

Not applicable

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