Original Research Article

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

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ABSTRACT

Cadmium is a toxic heavy metal which promotes oxidative stress in various organs including the brain. Restraint stress is a model of stress which involves physical constraint to induce homeostasis imbalance. This study aimed to evaluate the effects of restraint stress and cadmium administration on cerebral antioxidants and inflammation in female Wistar rats. 24 female Wistar rats (180-220g) were randomly divided into 4 groups (n=6 each): Control (CTL), Restraint stress alone (RSS), Cadmium alone (CCC), Cadmium + Restraint stress (RSC).The experimental groups were subjected to cadmium chloride 100mg/kg b.w. orally and restraint stress for 30 minutes using wire mesh. 24 hours post last cadmium administration and restraint stress exposure, all animals were anaesthetized and sacrificed. The brain was excised, homogenized and analyzed for antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), lipid peroxidation (Malondialdehyde). Results showed that there was significant ($p<0.05$) decrease in superoxide dismutase in cadmium alone and restraint stress alone groups when compared to control. The findings revealed that was a significant ($p<0.05$) decrease in cerebral catalase in both restraint stress alone and Cadmium alone groups when compared to Control. Additionally, a signficant ($p<0.05$) decrease in catalase activity was observed in cadmium + restraint group when

compared to restraint stress alone and Cadmium alone groups. Furthermore, cadmium exposure led to a significant ($p<0.05$) decrease in cerebral glutathione peroxidase when compared to control. Additionally, combined exposure to cadmium and restraint stress significantly ($p<0.05$) decreased cerebral glutathione peroxidase when compared to restraint stress and Cadmium alone groups. In the cadmium alone group, there was significant ($p<0.05$) increase in Malondialdehyde when compared to the control group. In conclusion, this present study have shown that combined exposure to cadmium and restraint stress increased free radical production and significantly decreased antioxidant system indicating that combined mechanism of both factors adversely induced neurological damage.

Keywords: Cadmium chloride, restraint stress, oxidative stress, antioxidant enzymes, lipid peroxidation.

INTRODUCTION

Stress is any imbalance in homeostasis caused by either ~~extrinisic~~extrinsic or intrinsic stressor (Devi *et al*., 2019). Stress is regulated by the coordinated interaction between the nervous, endocrine and immune system through the hypothalamic-pituitary adrenal (HPA) axis and the sympathetic adreno-medullar (SAM) axis (Mifsud and Reul, 2018). Restraint stress is an experimental model that mimics human stress experience in animals (Van *et al*., 2022). Rats are exploratory animals, confining and immobilizing them can induce both physical and psychological stress (Oluwatobi *et al*., 2024). Research has established a link between repetitive stress, oxidative stress and inflammation dysregulation (Czarny *et al*., 2018).

One of the major causes of environmental toxicity globally is heavy metal, this is due to increase urbanization, industrialization and indiscriminate disposal of waste products containing heavy metals (Genchi *et al*., 2020). Cadmium (Cd) is a heavy metal which occurs naturally in the earth crust. There is increased bioavailability of Cd in the environment due to increase anthropogenic activities such as electroplating, battery production and sludge fertilization (Hayat *et al*., 2019). Cadmium is known to exert toxicological effect on various organs system including the kidney, liver, heart, and brain (Bhattacharyya *et al*., 2023). Humans are exposed to cadmium either through ingestion of contaminated food and water or inhalation of contaminated air. Cadmium gains entry into the nervous system via the blood brain barrier accumulating in the brain, resulting to alteration in several neurological functions (Arruebarrena et al., 2023). Cadmium exposure has also been linked to mitochrondrial dysfunction in cerebral neurons

resulting in oxidative imbalance (Li *et al*., 2024). Hence, this study seeks to evaluate the effect of restraint stress and cadmium chloride administration on cerebral antioxidant in female Wistar rats.

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2.0 Material and methods

2.1 Chemical and compounds

Cadmium chloride was purchased from kermel, china. Chloroform, Normal Saline, distilled water, Buffered formalin, phosphate buffer saline was purchased from department of science laboratory, LAUTECH, Oyo, Nigeria.

2.2 Experimental Protocol and Animals

Twenty-four (24) female rats (180-220g) were used for the study. The rats were kept in a ~~well ventilated~~well-ventilated animal house (12/12 hour light and dark cycle) of the department of physiology, Ladoke Akintola University of Technology. The rats were acclimatized for two weeks and had unrestricted access to clean water and feed before the experiment. 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. After acclimatization, the rats were divided randomly into four groups with six (6) rats in each group and the experiment lasted for 21 days.

Group I = Control group (CTL), were given feed and water *ad* libitum.

Group II= Restraint Stress Alone (RSS), were subjected to restraint stress using wire mesh for 30 minutes daily.

Group III= Cadmium Alone (CCC), were administered cadmium chloride (100mg/kg/b.w) orally

Group IV= Cadmium+ Restraint stress (RSC) received cadmium chloride (100mg/kg/b.w) and were subjected to restraint stress for 30 minutes.

2.3 Collection and Preparation of samples

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Twenty four hours post administration; rats were anesthetized by placing each rat in a dessicator containing cotton wools soaked with chloroform. Brain organ was excised and divided into two parts. The first part was homogenized using mortar and pestle on cold ice. Then, it was centrifuged at 5000rpm for ten minutes. The homogenate was assayed for biochemical analysis. The second part of the brain was used for histological examination.

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2.4 Biochemical Tests

Glutathione perioxide (GPx), catalase (CAT), malondialdehyde (MDA), and superoxide dismutase (SOD) activities in brain tissues were evaluated spectrophotometrically in accordance with the enclosed pamphlets attached to commercial kits purchased from Biodiagnostic (Cairo, Egypt),

2.5 Statistical Analysis

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.

3.0 Results and Discussion

Results

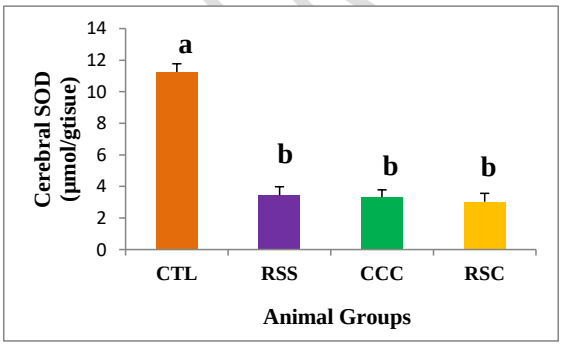


Figure 1: Effect of restraint stress and cadmium chloride administration on cerebral superoxide dismutase in female Wistar rats.

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 significant ($p<0.05$) decrease in SOD levels in RSS, CCC, and RSC group when compared to CTL. ~~However~~However, there was no significant difference in RSC when compared to RSS and CCC.

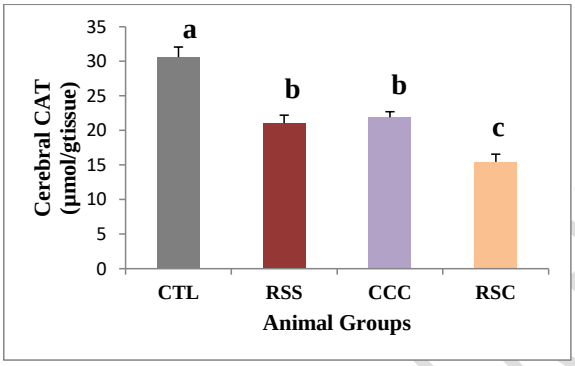


Figure 2: Effect of restraint stress and cadmium chloride administration on cerebral catalase in female Wistar rats.

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 a significant ($p<0.05$) decrease in cerebral catalase in both RSS and CCC groups when compared to CTL. Additionally, a significant ($p<0.05$) decrease in catalase activity was observed in RSC when compared to RSS and CCC groups.

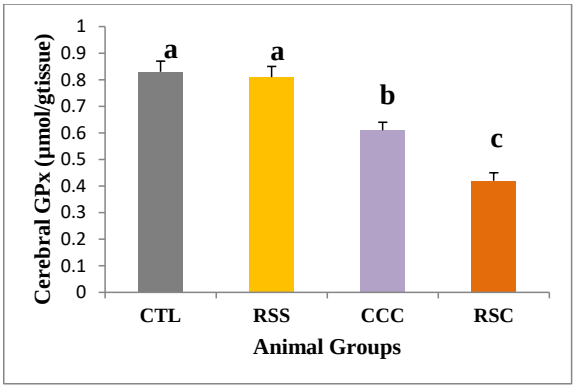


Figure 3: Effect of restraint stress and cadmium chloride administration on cerebral glutathione peroxidase (GPx) in female Wistar rats.

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.

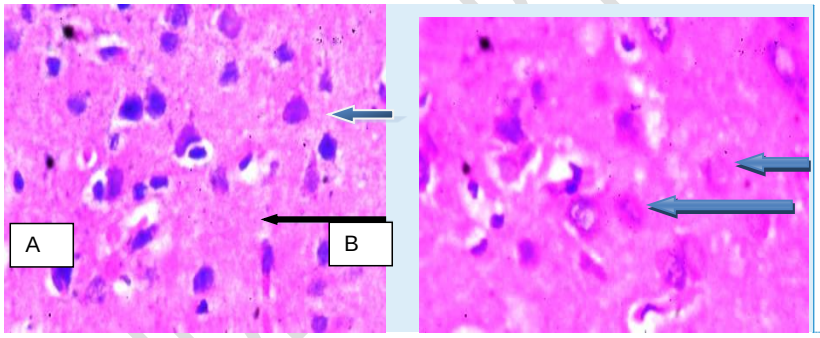
There was significant ($p<0.05$) decrease in cerebral glutathione peroxidase in CCC group when compared to CTL. There was significant ($p<0.05$) decrease in RSC when compared to RSS and CCC groups.

Table 1: Effect of restraint stress and cadmium chloride administration on cerebral malondialdehyde in female Wistar rats.

Cerebral MDA (nmol/g tissue)	CTL	RSS	CCC	RSC
	25.38±1.11[a]	26.24±1.18[a]	32.59±1.64[b]	33.49±1.99[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.

In female Wistar rats exposed to CCC group there was significant ($p<0.05$) increase in MDA level when compared to CTL. However, there was no significant difference in RSS group when compared to CTL. In the RSC group there was no significant difference when compared to the RSS and CCC groups.



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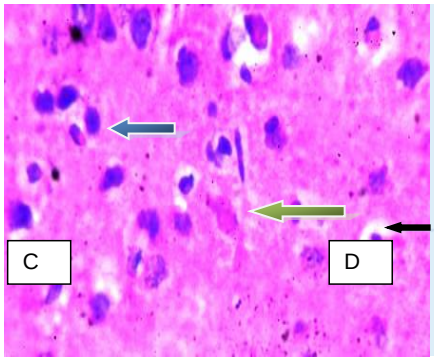

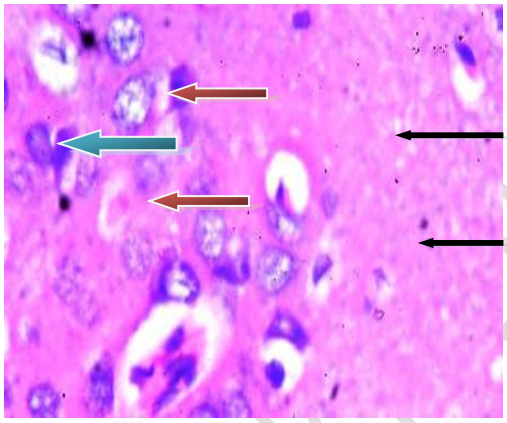

Figure 4: Effect of cadmium chloride administration and restraint stress on cerebral histology in female Wistar rats.

Haematoxylin and Eosin stained photomicrographs of the cerebrum of control and experimental rats. Histological sections of CTL rats (A) and RSS rats (B) showed a normal neuronal (blue arrow),capillaries (red arrow), and stroma (black arrow) while in the CCC (C) rats the cerebrum showed several normal neuronal cells (blue arrow), few neuronal cells with moderate necrosis (green arrow), mildly dilated capillaries (black arrow), and the stroma appear normal (black arrow). In the RSC (D), cerebrum with normal neuronal cells (blue arrow), few depleted neuronal cells (red arrow), capillaries and stroma appear normal (black arrow) was seen (H & E, ×400).

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Discussion

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Various researches have established that cadmium toxicity induced oxidative stress in animals and human (El-Habit *et* al., 2014; Agnihotri et al., 2015; Genchi *et al*., 2020). Chronic stress has been linked to oxidative stress (Juszczk *et al*., 2021). The brain is particularly vulnerable to oxidative stress, any form of disruption in this organ have a serious impact on the entire body. Antioxidant enzyme such as superoxide dismutase and Catalase protects cells against oxidative damage caused reactive oxygen species (ROS) (Ighodaro and Akinloye, 2018). In fig 1 and 2, results observed in the restraint stress alone and cadmium alone groups is in-line with the previous studies of Zafir and Banu, (2009); Alnahdi and Sharaf, (2019) where there was significant ($p<0.05$) decrease in cerebral superoxide dismutase (SOD) and catalase (CAT) levels following cadmium intoxication and stress induction when compared to control. Previous studies have shown that cadmium and restraint stress independently can increase the production of reactive oxygen species (ROS) resulting in the depletion of antioxidant system. Cadmium disrupts cellular defense system by depleting antioxidant levels and increasing accumulation of ROS which can result in lipid peroxidation (Unsal *et al*., 2020). Additionally, cd can replace some essential elements like zinc and iron which are necessary for SOD and CAT activities and thus, decrease its antioxidant capacity (Arruebarrena et al., 2023). Exposure to repetitive stress leads to hyperactivation of the hypothalamic pituitary adrenal axis and sludge in catecholamine release, this increase in glucocorticoid and catecholamine hormones promotes oxidative metabolism thereby increasing ROS production and decreasing the SOD and CAT levels (Chainy and Sahoo, 2020). The combination of cadmium and restraint stress when compared to cadmium alone and restraint stress alone groups showed no significant difference in SOD level suggest that the level of SOD might have been reduced to a threshold by either cadmium alone or stress alone. However, the combined effect of cadmium and restraint stress further decreased the Catalase level suggesting both cadmium and stress exacerbate oxidative stress.

Glutathione peroxidase (GPx) is an enzymatic anti-oxidant that functions to protect cells from oxidative stress by catalyzing the reduction of hydrogen peroxide (H_2O_2) and lipid peroxide into oxygen and water (Carmo de Carvalho e Martins et al, 2022). Result observed in fig 3 is consistent with the previous research of Shagirtha et al., (2017), where cadmium alone group showed a significant ($p<0.05$) decrease

in cerebral glutathione peroxidase when compared to control. This is indicating that overproduction of free radicals in cadmium exposed rats caused oxygen oxidative damages to membrane lipid and protein which leads to decrease in antioxidant enzymes glutathione peroxidase (Shagirtha et al., 2017). The combined exposure to cadmium and restraint stress led to a further reduction in glutathione peroxidase activity, indicating that both factors intensify oxidative stress.

Malondialdehyde is the byproduct of lipid peroxidation, this occurs when oxidative stress damages cell membranes (Calyniuk et al., 2016). In table 1, elevated level of MDA observed in the cadmium alone group is consistent with the findings of Ojo et al. (2023) where cadmium alone group was significantly ($p<0.05$) increased when compared to control. This is likely due to the production of reactive oxygen species (ROS) such as peroxyl, superoxide and hydroxyl radicals. Indirectly, cadmium exposure generates these radicals by disrupting mitochrondrial function which stimulates lipid peroxidation by producing endoperoxidases through non-enzymatic cyclization reaction (Kaur *et al*., 2014). The combination of restraint stress and cadmium showed no statistical significant difference when compared to the cadmium and restraint stress alone group. This suggests that cadmium alone might have caused maximal effect on lipid peroxidation in which restraint stress may not cause further increase.

Heavy metals have been associated with significant histological changes in the brain tissues. In this present study, cerebral cortex section examination by light microscope, showed no notable differences among rats in control and restraint alone groups. Cadmium intoxication induced histological changes in the cerebral tissue including mild neuronal necrosis and capillaries dilation. This is consistent with the findings of Gok *et al*., (2022). Additionally, combined exposure to cadmium and restraint stress led to neuronal degeneration in the cerebrum.

4.0 CONCLUSION

In conclusion, this present study have shown that combined exposure to cadmium and restraint stress increased free radical production and significantly decreased antioxidant system indicating that combined mechanism of both factors adversely induced neurological 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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