Original Research Article

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

ABSTRACT

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. This study aimed to evaluate the effects of restraint stress and cadmium administration on kidney antioxidants in female Wistar rats. 24 female Wistar rats (180-220 g) 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 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 were excised, homogenized and analyzed for antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), lipid peroxidation (Malondialdehyde). The 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 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. 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 evaluated the combined effects of restraint stress and cadmium chloride administration on kidney antioxidants in female Wistar rats.

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 100 mg/kg/b.w. of cadmium chloride daily for 21 days according to Owolabi et al. (2024).

GROUP 4: Cadmium chloride + Restraint stress (RSC). The rats were orally administered with 100 mg/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 *post hoc* test for multiple comparisons. P value < 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Body Weights

Table 1. Body weight in control and experimental rats.

Parameters	CTL	RSS	CCC	RSC
Body weight (grams)	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). Data are expressed as mean ± SEM (n= 6). Values not sharing common superscript letters differ significantly at *p<0.05* while values with superscript of same letters are not significantly different.

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.

3.2. Renal Antioxidants

3.2.1 Renal superoxide dismutase

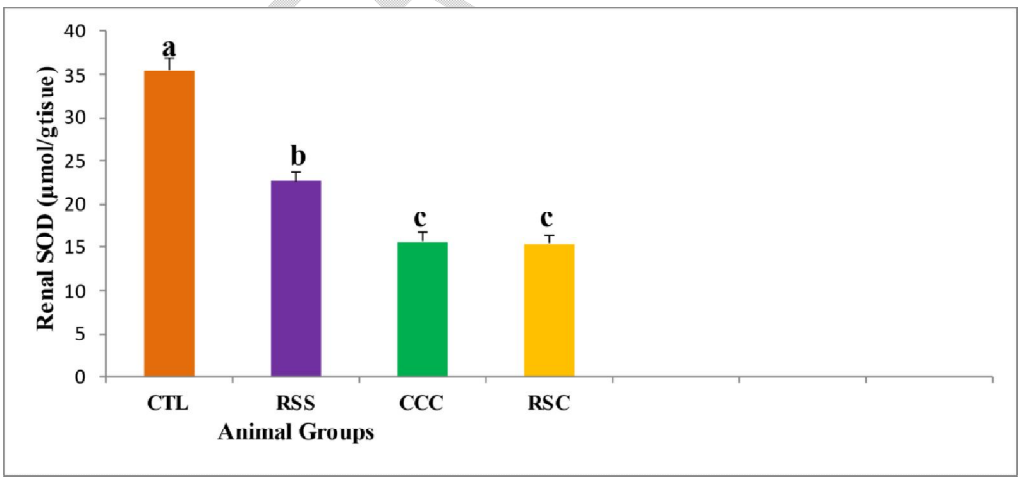

Figure 1: Effect of Cadmium chloride administration and restraint stress on renal superoxide dismutase in female Wistar rats.

Data are expressed as mean ± SEM (n= 6). Values not sharing common superscript letters differ significantly at *p<0.05* while values with superscript of same letters are not significantly different.

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.

3.2.2 Renal catalase

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 in the renal CAT level in the RSC when compared to RSS group but was significantly (p<0.05) increased when compared to CCC groups.

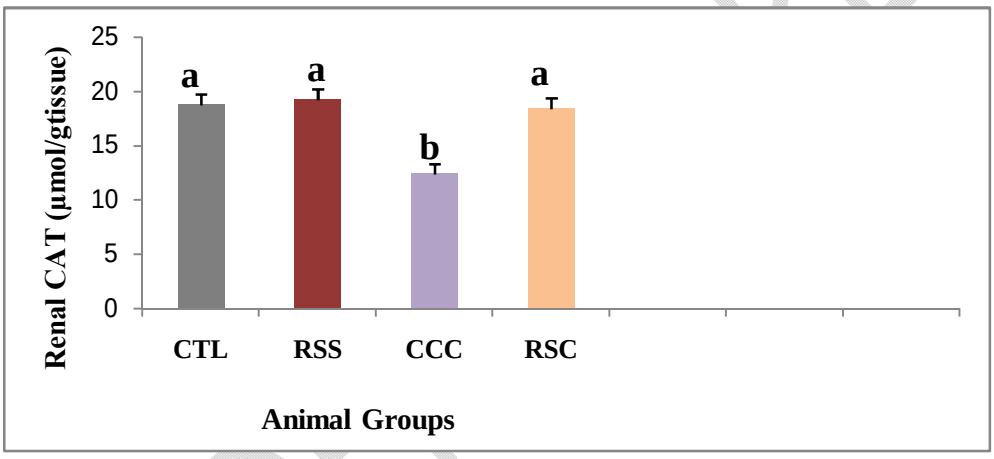

Figure 2: Effect of Cadmium chloride administration and restraint stress on renal catalase in female Wistar rats.

Data are expressed as mean ± SEM (n= 6). Values not sharing common superscript letters differ significantly at *p<0.05* while values with superscript of same letters are not significantly different.

3.2.3 Renal Glutathione Peroxidase

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.

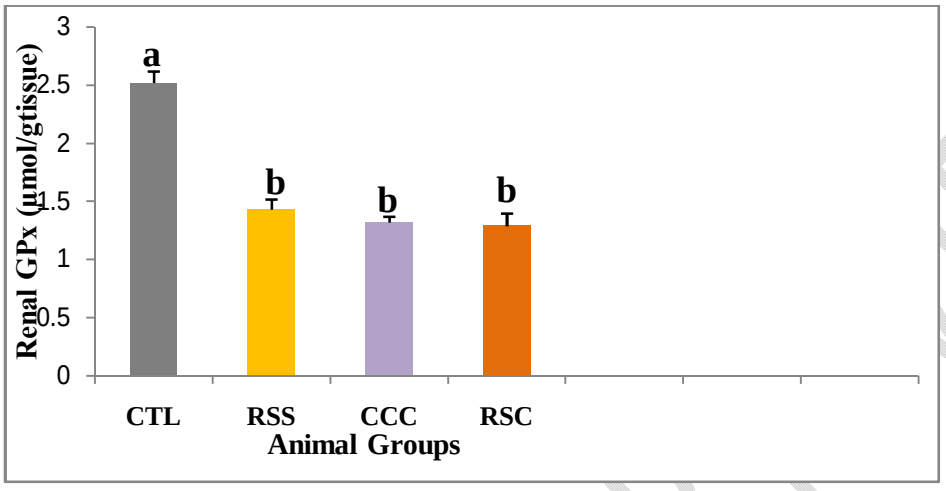

Figure 3: Effect of Cadmium chloride administration and restraint stress on renal glutathione peroxidase in female Wistar rats.

Data are expressed as mean ± SEM (n= 6). Values not sharing common superscript letters differ significantly at *p<0.05* while values with superscript of same letters are not significantly different.

3.2.4 Renal Malondialdehyde

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

Parameters	CTL	RSS	CCC	RSC
Renal MDA (nmol/g tissue)	18.27 ± 1.18^{a}	25.72 ± 1.56^{b}	35.38 ± 2.11^{c}	41.34 ± 1.57^{d}

Data were presented as mean ± SEM (n= 6). Means with different superscript letters indicates a statistically significant difference ($p<0.05$) while means with superscript of same letters are not significantly different.

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.

3.2.5 Renal Nitric Oxide

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

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

Data were presented as mean ± SEM (n= 6). Means with different superscript letters indicates a statistically significant difference ($p<0.05$) while means with superscript of same letters are not significantly different.

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 increased 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 sensitive 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. 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, H2O2, 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 significance 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). 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 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.

Ethical approval

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.

REFERENCES

Almeer, R.S., AlBasher, G.I., Alarifi, S., Alkahtani, S., Ali, D. and Abdel Moneim, A.E., 2019. Royal jelly attenuates cadmium-induced nephrotoxicity in male mice. *Scientific reports*, *9*(1), p.5825. https://doi.org/10.1152/physrev.00024.2019.

Andjelkovic, M., Buha Djordjevic, A., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D. and Bulat, Z., 2019. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. *International journal of environmental research and public health*, *16*(2), p.274.https://doi.org/10.3390/ijerph16020274.

Anjum, N.A., Sharma, P., Gill, S.S., Hasanuzzaman, M., Khan, E.A., Kachhap, K., Mohamed, A.A., Thangavel, P., Devi, G.D., Vasudhevan, P. and Sofo, A., 2016. Catalase and ascorbate peroxidase—representative H 2 O 2-detoxifying heme enzymes in plants. *Environmental science and pollution research*, *23*, pp.19002-19029.https://link.springer.com/article/10.1007/s11356-016-7309-6

Battin, E.E. and Brumaghim, J.L., 2009. Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell biochemistry and biophysics*, *55*(1), pp.1-23. https://link.springer.com/article/10.1007/S12013-009-9054-7

Bautista, C.J., Arango, N., Consuelo, P., Mitre-Aguilar, I.B., Trujillo, J. and Ramírez, V., 2024. Mechanism of cadmium-induced nephrotoxicity. *Toxicology*, p.153726. https://doi.org/10.1016/j.tox.2024.153726

Chen, Y., Feng, X., Hu, X., Sha, J., Li, B., Zhang, H. and Fan, H., 2018. Dexmedetomidine ameliorates acute stress□induced kidney injury by attenuating oxidative stress and apoptosis through inhibition of the ROS/JNK signaling pathway. *Oxidative medicine and cellular longevity*, *2018*(1), p.4035310. https://doi.org/10.1155/2018/4035310

Daenen, K., Andries, A., Mekahli, D., Van Schepdael, A., Jouret, F. and Bammens, B., 2019. Oxidative stress in chronic kidney disease. *Pediatric nephrology*, *34*, pp.975-991. https://doi.org/10.1155/2018/4035310

Ebrahimi, M., Khalili, N., Razi, S., Keshavarz-Fathi, M., Khalili, N. and Rezaei, N., 2020. Effects of lead and cadmium on the immune system and cancer progression. *Journal of Environmental Health Science and Engineering*, *18*, pp.335-343.https://link.springer.com/article/10.1007/s40201-020-00455-2

EL-Hengary, S.B.A., Abushofa, F.A. and Azab, A.E., 2023. Cadmium Toxicity: Insight into Sources, Toxicokinetics, and Effect on Vital Organs and Embryos. https://gsarpublishers.com/wp-content/uploads/2023/04/GSARJMS352023-Gelary-script.pdf

Erboga, M., Kanter, M., Aktas, C., Sener, U., Fidanol Erboga, Z., Bozdemir Donmez, Y. and Gurel, A., 2016. Thymoquinone ameliorates cadmium-induced nephrotoxicity, apoptosis, and oxidative stress in rats is based on its anti-apoptotic and anti-oxidant properties. *Biological trace element research*, *170*, pp.165-172. https://doi.org/10.1007/s12011-015-0453-x

Genchi, Giuseppe, Maria Stefania Sinicropi, Graziantonio Lauria, Alessia Carocci, and Alessia Catalano. "The effects of cadmium toxicity." *International journal of environmental research and public health* 17, no. 11 (2020): 3782. https://doi.org/10.3390/ijerph17113782

Hayat, Malik Tahir, Muhammad Nauman, Nida Nazir, Shafaqat Ali, and Nazneen Bangash. "Environmental hazards of cadmium: past, present, and future." In *Cadmium toxicity and tolerance in plants*, pp. 163-183. Academic Press, 2019.https://doi.org/10.1016/B978-0-12-814864-8.00007-3

Imenez Silva, P.H. and Mohebbi, N., 2022. Kidney metabolism and acid–base control: back to the basics. *Pflügers Archiv-European Journal of Physiology*, *474*(8), pp.919-934. https://doi.org/10.1007/s00424-022-02696-6

Jagaba, A.H., Lawal, I.M., Birniwa, A.H., Affam, A.C., Usman, A.K., Soja, U.B., Saleh, D., Hussaini, A., Noor, A. and Yaro, N.S.A., 2024. Sources of water contamination by heavy metals. In *Membrane Technologies for Heavy Metal Removal from Water* (pp. 3-27). CRC Press. https://www.taylorfrancis.com/chapters/edit/10.1201/9781003326281-2/sources-water-contamination-heavy-metals-ahmad-hussaini-jagaba-ibrahim-mohammed-lawal-abdullahi-haruna-birniwa-augustine-chioma-affam-abdullahi-kilaco-usman-usman-bala-soja-dalhatu-saleh-abdulmalik-hussaini-azmatullah-noor-nura-shehu-aliyu-yaro

Koushki, M., Farahani, M., Parsamanesh, N., Chiti, H., Fridoni, M.J., Chahkandi, S., Amiri-Dashatan, N. and Rezaei-Tavirani, M., 2024. Cadmium-induced Alterations in the Expression Profile of MicroRNAs: A Comprehensive Review. *Gene Expression*, (000), pp.0-0. https://doi.org/10.14218/GE.2023.00126

Kraft, P. and Kraft, B., 2021. Explaining socioeconomic disparities in health behaviours: A review of biopsychological pathways involving stress and inflammation. *Neuroscience & Biobehavioral Reviews*, *127*, pp.689-708. https://doi.org/10.1016/j.neubiorev.2021.05.019

Liu, Q., Zhang, R., Wang, X., Shen, X., Wang, P., Sun, N., Li, X., Li, X. and Hai, C., 2019. Effects of sub-chronic, low-dose cadmium exposure on kidney damage and potential mechanisms. *Annals of translational medicine*, *7*(8).http://doi.org/10.21037/atm.2019.03.66

Lubos, E., Loscalzo, J. and Handy, D.E., 2011. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities.https://doi.org/10.1089/ars.2010.3586

Masoomi, K.M., Jafari, S.M., Zaree, M.A., Jafari, S.A. and Khatibi, S.R., 2012. Effect of acute toxicity of cadmium in mice kidney cells. http://ijt.arakmu.ac.ir/article-1-148-en.html

Micali, A., Pallio, G., Irrera, N., Marini, H., Trichilo, V., Puzzolo, D., Pisani, A., Malta, C., Santoro, G., Laurà, R. and Santoro, D., 2018. Flavocoxid, a Natural Antioxidant, Protects Mouse Kidney from Cadmium□Induced Toxicity. *Oxidative medicine and cellular longevity*, *2018*(1), p.9162946. https://doi.org/10.1155/2018/9162946

Ovsiannikova, Y., Pokhilko, D., Kerdyvar, V., Krasnokutsky, M. and Kosolapov, O., 2024. Peculiarities of the impact of stress on physical and psychological health. *Multidisciplinary Science Journal*, *6*. ttps://10.31893/multiscience.2024ss0711

Papi, S., Ahmadizar, F. and Hasanvand, A., 2019. The role of nitric oxide in inflammation and oxidative stress. *Immunopathologia Persa*, *5*(1), pp.e08-e08. DOI:10.15171/ipp.2019.08

Prozialeck WC, Edwards JR. Mechanisms of cadmium-induced proximal tubule injury: new insights with implications for biomonitoring and therapeutic interventions. J Pharmacol Exp Ther 2012;343(1):2-12

Qu, N., He, Y., Wang, C., Xu, P., Yang, Y., Cai, X., Liu, H., Yu, K., Pei, Z., Hyseni, I., et al. (2020). A POMC-originated circuit regulates stress induced hypophagia, depression, and anhedonia. Mol. sychiatr. 25, 1006–1021. https://www.nature.com/articles/s41380-019-0506-1

Samarghandian, S., Azimi-Nezhad, M., Farkhondeh, T. and Samini, F., 2017. Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. *Biomedicine & Pharmacotherapy*, *87*, pp.223-229. https://doi.org/10.1016/j.biopha.2016.12.105

Song, J. and Choi, S.Y., 2023. Arcuate Nucleus of the Hypothalamus: Anatomy, Physiology, and Diseases. *Experimental Neurobiology*, *32*(6), p.371. doi:10.5607/en23040

Srivastava, K.K. and Kumar, R., 2015. Stress, oxidative injury and disease. *Indian Journal of Clinical Biochemistry*, *30*(1), pp.3-10. https://link.springer.com/article/10.1007/s12291-014-0441-5

Su, G., Song, H., Lanka, V., Liu, X., Fang, F., Valdimarsdóttir, U.A. and Carrero, J.J., 2021. Stress related disorders and the risk of kidney disease. *Kidney international reports*, *6*(3), pp.706-715. https://doi.org/10.1016/j.ekir.2020.12.032

Van Wyk, M., 2022. *Establishing and validating an in vivo rodent model of chronic restraint stress* (Doctoral dissertation, Stellenbosch: Stellenbosch University).http://hdl.handle.net/10019.1/126118

Xu, X., Nie, S., Ding, H. and Hou, F.F., 2018. Environmental pollution and kidney diseases. *Nature Reviews Nephrology*, *14*(5), pp.313-324. https://www.nature.com/articles/nrneph.2018.11

Yang, H. and Shu, Y., 2015. Cadmium transporters in the kidney and cadmium-induced nephrotoxicity. *International journal of molecular sciences*, *16*(1), pp.1484-1494. https://doi.org/10.3390/ijms16011484

Vieira, J. O., Duarte, J. O., Costa-Ferreira, W., Morais-Silva, G., Marin, M. T., & Crestani, C. C. (2018). Sex differences in cardiovascular, neuroendocrine and behavioral changes evoked by chronic stressors in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *81*, 426-437. https://doi.org/10.1016/j.pnpbp.2017.08.014

Duranova, H., Martiniakova, M., Omelka, R., Grosskopf, B., Bobonova, I., & Toman, R. (2014). Changes in compact bone microstructure of rats subchronically exposed to cadmium. *Acta Veterinaria Scandinavica*, *56*, 1-8.https://link.springer.com/article/10.1186/s13028-014-0064-0

He, S., Zhuo, L., Cao, Y., Liu, G., Zhao, H., Song, R., & Liu, Z. (2020). Effect of cadmium on osteoclast differentiation during bone injury in female mice. *Environmental toxicology*, *35*(4), 487-494. https://doi.org/10.1002/tox.22884

Haeri, V., Karimi, E., & Oskoueian, E. (2023). Synthesized nanoliposome□encapsulated kaempferol attenuates liver health parameters and gene expression in mice challenged by cadmium□induced toxicity. *Biotechnology and Applied Biochemistry*, *70*(1), 429-438. https://doi.org/10.1002/bab.2368

Jomova, K., Alomar, S.Y., Alwasel, S.H., Nepovimova, E., Kuca, K. and Valko, M., 2024. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of Toxicology*, *98*(5), pp.1323-1367. https://link.springer.com/article/10.1007/s00204-024-03696-4

Nwogueze, B.C., Ofili, I.M., Nnama, T.N. and Aloamaka, C.P., 2023. Oxidative stress-induced by different stressors alters kidney tissue antioxidant markers and levels of creatinine and urea: the fate of renal membrane integrity. *Scientific Reports*, *13*(1), p.13309. https://www.nature.com/articles/s41598-023-40454-5

Samarghandian, S., Farkhondeh, T., Samini, F. and Borji, A., 2016. Protective effects of carvacrol against oxidative stress induced by chronic stress in rat's brain, liver, and kidney. *Biochemistry research international*, *2016*(1), p.2645237. https://doi.org/10.1155/2016/2645237

Abdulrauf, R.A., Dawud, F.A., Emmanuel, N.S., Muhammad, H.D., Dange, A.S., David, B.A., Ogweje, A.E., Alexander, A.U. and Yahuza, M., 2018. Lipid peroxidation and some antioxidant enzymes evaluation in apple cider vinegar (ACV) treated male and female wistar rats exposed to chronic restraint stress. *Advances in Enzyme Research*, *6*(3), pp.21-28. **DOI:** 10.4236/aer.2018.63003

Samarghandian, S., Azimi-Nezhad, M., Farkhondeh, T. and Samini, F., 2017. Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. *Biomedicine & Pharmacotherapy*, *87*, pp.223-229.https://doi.org/10.1016/j.biopha.2016.12.105

Ogunrinola, O.O., Wusu, D.A., Fajana, O.O., Olaitan, S.N., Smith, Z.O. and Bolaji, A.R.I., 2016. Effect of low level cadmium exposure on superoxide dismutase activity in rat. *Tropical Journal of Pharmaceutical Research*, *15*(1), pp.115-119. **DOI:**10.4314/tjpr.v15i1.16

Owolabi, G. O., Jejelaye, E. A., & Offiong, I. E. (2024). Effect of Cadmium Chloride Administration and Restraint Stress Model on Cardiac Function of Female Wistar Rats. *Asian Journal of Biology*, *20*(12), 88–99. https://doi.org/10.9734/ajob/2024/v20i12463

Poli, V., Madduru, R., Aparna, Y., Kandukuri, V. and Motireddy, S.R., 2022. Amelioration of cadmium-induced oxidative damage in wistar rats by Vitamin C, zinc and N-acetylcysteine. *Medical Sciences*, *10*(1), p.7.https://doi.org/10.3390/medsci10010007

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A. and Catalano, A., 2020. The effects of cadmium toxicity. *International journal of environmental research and public health*, *17*(11), p.3782. https://doi.org/10.3390/ijerph17113782

Do Vale, G.T., Pereira, B.P., Potje, S.R. and Ceron, C.S., 2023. Nitric oxide (NO) donors in kidney damage and diseases. In *Nitric Oxide in Health and Disease* (pp. 213-230). Academic Press. https://doi.org/10.1016/B978-0-443-13342-8.00010-7

Kukongviriyapan, U., Pannangpetch, P., Kukongviriyapan, V., Donpunha, W., Sompamit, K. and Surawattanawan, P., 2014. Curcumin protects against cadmium-induced vascular dysfunction, hypertension and tissue cadmium accumulation in mice. *Nutrients*, *6*(3), pp.1194-1208. https://doi.org/10.3390/nu6031194

Bernatova, I., Puzserova, A., Balis, P., Sestakova, N., Horvathova, M., Kralovicova, Z. and Zitnanova, I., 2018. Chronic stress produces persistent increases in plasma corticosterone, reductions in brain and cardiac nitric oxide production, and delayed alterations in endothelial function in young prehypertensive rats. *Frontiers in physiology*, *9*, p.1179. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2018.01179/full

Modlinger, P.S., Wilcox, C.S. and Aslam, S., 2004, July. Nitric oxide, oxidative stress, and progression of chronic renal failure. In *Seminars in nephrology* (Vol. 24, No. 4, pp. 354-365). WB Saunders. https://doi.org/10.1016/j.semnephrol.2004.04.007

Matović, V., Buha, A., Đukić-Ćosić, D. and Bulat, Z., 2015. Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. *Food and Chemical Toxicology*, *78*, pp.130-140. https://doi.org/10.1016/j.fct.2015.02.011