EFFECT OF AGE ON COLONIC DAMAGE INDUCED BY CADMIUM EXPOSURE IN FEMALE WISTAR RATS

ABSTRACT

BACKGROUND: Cadmium, a known genotoxic metal, has been shown to worsen colonic damage in both animal models and human studies. Research has indicated that cadmium accumulation in the colon results in mucosal damage, inflammation, disrupted immune responses, and an increased risk of gastrointestinal diseases. However, the relationship between age-related changes in the colon and cadmium exposure is not well understood. This study aimed to examine how aging affects colonic damage caused by cadmium exposure in female Wistar rats.

METHOD: Forty eight (48) female Wistar rats were allotted for this study. They were divided into six groups with eight animals each as follows: Group one: control group for young (6 weeks old) female Wistar rats, group two**:**cadmium exposed young (6 weeks old) female Wistar rats, group three: control group for middle age (10 weeks old) female Wistar rats, group four: cadmium exposed middle age (10 weeks old) female Wistar rats, group five: control group for old (24 weeks old) female Wistar rats group six: cadmium exposed old (24 weeks old) female Wistar rats. Cadmium exposed animals were exposed to 50mg/kg body weight of $CdCl_2$ orally for 28 days and Animals in control groups received 0.5 ml of distilled water each ensuring uniformity and consistency of exposure across the experimental groups.The animals were weighed daily throughout the exposure period.Rats were sacrificed on the 29th day by cervical dislocation. The colon was excised and the distal portion was homogenized and assessed for antioxidants level [Superoxide Dismutase (SOD), catalase (CAT), reduced glutathione (rGSH)],oxidative stress markers **[**8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA)] spectrophotometrically, and inflammatory marker [tumor necrosis factor (TNF-α)] using ELISA. Histological evaluation was assessed from the mid portion of the colon using Hematoxylin and Eosin staining method. Total intestinal bacterial count was done microscopically after endoscopic biopsy.Data were analyzed using One Way Analysis of Variance (ANOVA) of Graphpad prism 5.0, Tukey's *Post-hoc* test was used for multiple comparison with statistical significance set at $P<0.05$.

RESULTS AND DISCUSSION: The body weight of rats significantly reduced in of Cd (young and middle-age) groups compared with their respective control groups (P=0.001; 0.05), but increased in Cd old age rats compared to Cd (young and middle-age) groups (P= 0.001). There was no significant difference in SOD activity among Cd groups, except a reduction in the old group compared to the middle-age group (P=0.001). Catalase activity and GSH levels decreased in both middle-age and old Cd groups (P=0.01; 0.05). MDA levels increased significantly while 8-OHDG levels decreased in the old group (P=0.01). TNF-α activity and total colonic bacterial count were significantly increased in the old Cd group compared to others (P=0.001; 0.001). Histological analysis showed different tissue damage patterns in the colon across Cd-exposed age groups, with more severe changes in older rats.

CONCLUSION: Exposure to cadmium exacerbates age related intestinal damage through increase in permeability, oxidative stress and impaired defense system.

Key words: Wistar Rats, Cadmium, Heavy Metal, Animal Models

INTRODUCTION

Cadmium is a widely distributed heavy metal found in the environment, primarily due to industrial, mining, and agricultural activities, posing severe health risks to both humans and animals (Orisakwe et al., 2006). Exposure to cadmium mainly occurs through ingestion of contaminated food and water, inhalation of polluted air, and sometimes through skin absorption (Wang et al., 2021). Once inside the body, cadmium accumulates in organs like the kidneys, liver, lungs, and intestines, leading to harmful effects, including oxidative stress, inflammation, and disruption of normal cellular functions (Sincropi et al., 2010).Over the past few decades, the use of chemicals both in industries and local settings has dramatically increased, resulting in heightened chemical exposure risks (Rafati &Moghadamnia, 2010; Rafati et al., 2015). Industrial chemicals are commonly found in products such as soaps, cosmetics, plastics, inks, cleaning agents, and other everyday materials used in homes and workplaces (Khalil et al., 2022). Many of these chemicals, including sulfuric acid, sodium hydroxides, nitrogen, mercury, lead, arsenic, propylene, ethylene, and cadmium, are categorized as heavy metals. Prolonged or excessive exposure to such chemicals has been associated with toxic effects on the human body (Weldeslassie et al., 2018).

Cadmium exposure through the gastrointestinal tract mainly occurs by consuming contaminated food and water. Smoking is another significant source of exposure; each cigarette contains about 1-2 mg of cadmium, leading smokers to have cadmium levels four to five times higher than non-smokers (Ali et al., 2019). Ingested cadmium is absorbed by up to 8%, while inhaled cadmium is absorbed by as much as 30%. Absorption increases when the diet is low in calcium, iron, and protein, while zinc can reduce cadmium absorption, possibly by promoting metallothionein synthesis. Cadmium is transported in the blood, bound to erythrocytes and large proteins like albumin, and a smaller portion is bound to metallothionein. Approximately 50-75% of the body's cadmium is stored in the liver and kidneys, and it is believed to have a half-life of 10-30 years (Jokanovic, 2010).The gastrointestinal (GI) tract, particularly the intestines, is highly susceptible to cadmium toxicity (Lech &Sadlik, 2015). The intestines play a critical role in nutrient absorption, immune regulation, and maintaining a barrier against harmful substances. Cadmium accumulation in the intestines can cause tissue damage and inflammation, triggering the recruitment of inflammatory cells like neutrophils (Zhou et al., 2001). Additionally, cadmium negatively affects gut microflora, which can further impact intestinal health (Duan et al., 2021). Besides direct cytotoxicity, cadmium also promotes carcinogenesis through the production of reactive oxygen species (ROS), which is a primary driver of inflammation (Lee et al., 2018). Elevated ROS levels can lead to oxidative stress and DNA damage (Xie et al., 2019). Cadmium exposure is also linked to the assembly of the protein P53, which induces apoptosis in intestinal epithelial cells (Lee et al., 2018).

The colon, a key part of the GI tract responsible for nutrient absorption and waste elimination, is particularly vulnerable to cadmium’s harmful effects (Azzouz & Sharma, 2018). Studies have

shown that cadmium accumulation in the colon leads to mucosal damage, inflammation, altered immune responses, and a higher risk of gastrointestinal diseases, including colorectal cancer (Tinkov et al., 2018). These findings highlight the critical importance of addressing cadmium exposure and its effects on colonic health.Age-related changes in the body, such as reduced antioxidant defenses and impaired cellular repair mechanisms, also increase vulnerability to environmental toxins, including cadmium (Bachman et al., 2020). With age, the gastrointestinal structure and function undergo changes, such as alterations in gut composition, weakening of the intestinal barrier, and shifts in immune responses. As a result, the colon's response to cadmium exposure may be influenced by aging (Mangiola et al., 2018).Despite well-documented evidence of cadmium's adverse effects on the colon, the impact of age on cadmium-induced colonic damage remains poorly understood. Previous studies have shown that cadmium accumulation can lead to mucosal damage, inflammation, and immune dysregulation in the colon, increasing susceptibility to colorectal cancer (Kumar et al., 2013). The aging process, with its associated changes in gut structure, barrier integrity, and immune responses, could potentially exacerbate these effects (Mangiola et al., 2018).

2. MATERIALS AND METHOD

2.1 Experimental Animals

Forty-eight (48) female Wistar rats at different age groups: sixteen young female Wistar rats at 4 weeks old, sixteen middle age female Wistar rats at 8 weeks old, and sixteen old female Wistar rats at 22 weeks old were purchased from the Experimental Animal Unit of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, and acclimatized for 14 days. After acclimatization there were sixteen young female Wistar rats at 6 weeks old, sixteen middle age female Wistar rats at 10 weeks old, and sixteen old female Wistar rats at 24 weeks old. The animals were kept in the Physiology Laboratory, LAUTECH, Ogbomoso under usual laboratory conditions at a temperature of 22±2 °C, relative humidity of 60%, 12-hr light-dark cycle, with free access to feed (Glory Vet Nig. Ltd., Ogbomoso, Nigeria), and clean tap water*ad libitum*.

2.2 Chemicals and Reagents Preparation

All chemicals and reagents used were of high analytical grade and were prepared under standard laboratory conditions. Cadmium chloride ($CdCl_2$) was purchased from Loba Chemie Pvt. Ltd India. Sucrose and formalin were obtained from Department of Chemistry, LAUTECH, Ogbomoso. Cadmium chloride solution was prepared by dissolving 50 mg of $CdCl_2$ in 250ml of distilled water. Sucrose solution was prepared by diluting 25g of sucrose in 25ml of distilled water for the preservation of colonic tissue for biochemical analysis. For the fixation of the excised colonic tissues for histological analysis, 10 % neutral buffered formalin was prepared by diluting 100ml of formalin in 900 ml of distilled water.

2.3 Study Design

Fifty eight female Wistar rats were allottedinto six groups of eighteen animals each as follows:

Group 1: Control group for young (6 weeks old) female Wistar rats.
Group 2: Cadmium exposed young (6 weeks old) female Wistar rats.
Group 3: Control group for middle age (10 weeks old) female Wistar rats.
Group 4:Cadmium exposed middle age (10 weeks old) female Wistar rats.
Group 5:Control group for old (24 weeks old) female Wistar rats.
Group 6:Cadmium exposed old (24 weeks old) female Wistar rats.
Cadmium exposed animals were exposed to 50mg/kg body weight of $CdCl_2$orally for 28 days and Animals in control groups received 0.5 mlof distilled water each ensuring uniformity and consistency of exposure across the experimental groups. Selected doses and duration of exposure of cadmium was based on previous report (Adegoke *et al.,* 2017).

2.4Tissue Collection and Preparation of Colon Homogenate

The distal colon of the rats measuring six (6) cm beginning from the proximal end of the rectum was excised. The excised distal colon was rinsed in saline and divided into three portions (proximal, mid and distal). The distal portion of the colon was preserved in 0.25M sucrose solution maintained 4ºC for biochemical assays. The mid portion of the colon was preserved in 10% formalin for histological analysis, while the proximal region was reserved for intestinal microbiota count analysis. The animals were weighed daily throughout the exposure period.

2.5Biochemical Analysis

Distal portion of colon in 0.25M sucrose solution was homogenized and centrifuged at 3000 rpm for 15 minutes. Colon (distal portion) homogenates was used to assess catalase (CAT) and Superoxide dismutase (SOD) activities, glutathione (GSH), Total antioxidant capacity (TAC), and total protein levels. Superoxide dismutase (SOD) and catalase activities were assessed spectrophotometrically by the method of Misra and Fridovich, (1972). Glutathione concentration, total antioxidant capacity (TAC) and total protein levels were assessed spectrophotometrically at 570-610 nm using the method of Sinha *et al.,* (2021). MDA concentration was evaluated according as described by Adegunlola*et al.* (2012). Oxidative DNA damage was measured by quantitative immunohistochemistry using a monoclonal antibody to 8-OHdG as described (Yarborough *et al.,* 1996). TNF-α was assessed using using Enzyme linked Immunsorbent assay (ELISA) kits, as instructed by the manufacturer (eBIOSCIENCE, Bender MedSystems GmbH, Wien, Austria). The fixed colon tissues were sectioned, after which stained with Hematoxylin and Eosin as previously described (Afolabi *et al*., 2022). The slides were observed and photomicrographs were taken at 400X magnification. Endoscopic biopsy was done on the proximal region of the excised colon as described by Tang *et al.* (2020) to count intestinal microbiota.

2.6 Statistical analysis

Data were presented as Mean ± standard error of the mean (Mean ± SEM) and analyzed using graph pad prism 5.0, One way analysis of variance (ANOVA). Tukey's post-hoc test was used for multiple comparisons. (P value) $P<0.05$ was considered statistically significant.

3.RESULTS

3.1 Effects of age on body weight of female wistar rats exposed to cadmium.

The result shows a significant reduction in the body weight of Cd (young and middle-age) groups only compared with their respective control groups (P=0.001; 0.05); however, there was a significant increase in Cd old age rats compared to Cd (young and middle-age) groups (P= 0.001).

3.2 Effect of age on colonic antioxidants activities (superoxide dismutase, catalase, glutathione), and total antioxidant capacity of cadmium exposed female Wistar rats.

There was no significant difference in the activity of SOD in the three Cd groups compared to their controls. However, significant reduction in superoxide dismutase activity was only seen in Cd old group compared to Cd middle-age group (P= 0.001).

Significant decrease in Catalase activity and GSH level was seen in Cd middle-age and Cd old age groups compared to their controls (P=0.01; 0.05). Also, a reduction in Catalase activity was seen in Cd middle-age and Cd old age groups compared to Cd young age group (P=0.001).

There was no significant difference in total antioxidant capacity (TAC) in various Cd groups when compared to their respective control and age groups.

3.3 Effect of age on oxidative stress markers (Malondialdehyde and 8-OHDG) of cadmium exposed female Wistar rats.

There was a significant increase in Malondialdehyde(MDA) level of Cd old groups compared to its control and other age (young and middle-age) groups (P=0.01; 0.05). No significant difference was seen in Cd (young and middle-age) groups compared to their respective control groups. Also, a significant reduction was seen in 8-OHDG activity in Cd old group compared to its control and Cd young and middle-age groups (P=0.001; 0.001; 0.05). However, no significant difference was seen in Cd young and middle-age groups when compared to their respective control groups.

3.4 Effect of Age on Colonic inflammatory marker (TNF-α concentration) of Cadmium exposed female rats

There was a significant increase in TNF-αactivity of Cd groups compared to their control groups (P= 0.001). Additionally, TNF-αactivity significant increase in Cd (old) age compared to Cd (young and middle-age) groups (P= 0.001).

3.5 Effect of Age on total Colonic bacterial count of Cadmium exposed female rats

Total colonic bacterial count significantly increased in Cd old groups compared to its control and other age (young and middle-age) groups (P=0.001; 0.01). No significant difference was seen in Cd (young and middle-age) groups compared to their respective control groups.

3.6: Effect of age on histology of the colon (H&E X100)in cadmium exposed female wistar rats.

Cadmium exposedyoung ratsulceration of the mucosa (blue arrow) with normal serous, muscular, and submucus layers compared to its control, Cadmium exposedmiddle-age rats showed normal serous, muscular, and mucus layerswith submucosal enlargement (green arrow) compared to its control, and Cadmium exposedold-age rats showed normal serous, muscular, submucus and mucus layers with arteriolar dilation (white arrow) compared to its control.

DISCUSSION

Cadmium is a widespread environmental pollutant linked to gastrointestinal dysfunction in both animal models and humans (Wang et al., 2012). Older individuals are particularly vulnerable to cadmium's adverse effects due to age-related physiological changes that influence metal absorption and toxicity (Bernard, 2008). Cadmium exposure in female Wistar rats induces significant toxicity and growth impairments, with the effects being age-dependent. Cd-exposed young rats show the most pronounced impact, weighing approximately 76.6% less than its control which may be as a result of severe gastrointestinal dysfunction, reduced food intake, and impaired nutrient absorption (Jacquillet*et al*, 2007). Additionally, early-life exposure may disrupt critical growth signals, further explaining the stark differences in weight gain (Men *et al*., 2021). Middle-aged and older rats also exhibit suppressed weight gain when exposed to cadmium, although to a lesser extent, suggesting that metabolic toxicity persists throughout life (Sataru*get al*., 2022). However, the effects may diminish with age due to possible physiological adaptations or existing dysfunctions. Nevertheless, cadmium remains harmful, but younger rats are most vulnerable to its effects.The study's results indicate that cadmium exposure significantly reduced SOD levels in older, but not in middle-age or young rats. There were significant reductions in colonic catalase levels with cadmium exposure in both middle-aged and old rats. Superoxide dismutase (SOD) and catalase are key enzymes protecting cells from oxidative stress. SOD converts superoxide radicals into hydrogen peroxide, while catalase breaks hydrogen peroxide down into water and oxygen. These enzymes function together to neutralize reactive oxygen species (ROS) and prevent damage to cellular components (Zinellu and Mangoni, 2021). SOD is located in the cytoplasm, mitochondria, or extracellular space, while catalase resides in peroxisomes. Their coordinated action ensures efficient ROS detoxification and maintains cellular redox balance (Zinellu and Mangoni, 2021). The observed decline of these antioxidants in Cd-exposed old and middle-aged rats reflects a weakened antioxidant defense system, suggesting disrupted redox homeostasis in the aging colonic mucosa, with more severe effects seen with advanced age.Cadmium inhibits the antioxidant activity of both SOD and catalase by displacing essential metal cofactors like zinc and copper ions, and binding to sulfhydryl groups (Ognjanović et al., 2008), reducing the enzymes' catalytic activities. Age-related nutritional deficiencies may exacerbate cadmium's inhibitory effects on catalase function in old rats. Additionally, oxidative damage accumulates over time, and chronic inflammation in aging tissues can impair antioxidant activity (Salminen et al., 2012). Older rats likely have reduced SOD reserves to buffer cadmium-induced oxidative stress, compared to middle-aged rats, which maintain SOD levels despite exposure.

Cadmium can also influence the gene expression and transcription factors responsible for antioxidant defense. Studies have shown cadmium exposure can suppress catalase and other antioxidant enzymes by interfering with transcriptional regulation (Valko et al., 2005). The significant reduction in catalase concentration in Cd-exposed middle-aged rats compared to controls may be due to cadmium-induced repression of catalase gene expression, compromising its synthesis and function (Hada et al., 2014).Glutathione, known as the "master antioxidant," is vital for defending cells against harmful molecules, detoxifying substances, maintaining redox

balance, and regenerating antioxidants like vitamins C and E (Teskey et al., 2018; Chakravorty et al., 2023). Glutathione (GSH) also neutralizes ROS, aids toxin excretion, enhances immune cell function, and indicates cellular health through its GSH/GSSG ratio (Chakravorty et al., 2023). Normally, GSH levels increase from young to middle age, boosting antioxidant defenses in response to age-related oxidative stress, with production peaking around maturity (Liu et al., 2011). However, cadmium exposure significantly decreased GSH in middle-aged and old rats compared to controls. Cadmium likely binds to and oxidizes GSH, either directly or by generating ROS (Ognjanović et al., 2008). Additionally, cadmium may inhibit GSH synthesis or deplete cofactors like selenium (Renugadevi and Prabu, 2010). The marked decline in colonic GSH in Cd-exposed old rats suggests a vulnerability linked to aging, as factors like reduced GSH synthesis with age, chronic inflammation, and mitochondrial dysfunction exacerbate cadmium-induced oxidative damage (Chung et al., 2009; Cuypers et al., 2010).

Malondialdehyde (MDA) levels, a marker of lipid peroxidation, significantly increased in Cd-exposed old rats compared to young and middle-aged rats. MDA is a well-established biomarker for oxidative stress (Khoubnasabjafari et al., 2016). Aging is associated with increased production of ROS and reduced antioxidant defenses, leading to elevated oxidative stress (Benz, 2008). Old rats likely experience more oxidative damage at baseline, which cadmium exposure exacerbates (Johri et al., 2010). Impaired mitochondrial function in aging also makes old rats more susceptible to cadmium-induced oxidative damage. Despite significant differences in antioxidant enzyme levels, total antioxidant capacity (TAC) remained unchanged across control and cadmium-treated rats of different ages. TAC reflects the collective action of all antioxidants, providing a comprehensive measure of the antioxidant response (Silvestrini et al., 2023).The lack of significant differences in TAC across groups may result from compensatory antioxidant mechanisms activated in response to oxidative stress. When faced with elevated ROS levels, the body may upregulate alternative antioxidant pathways to maintain redox balance (Franco et al., 2007). Non-enzymatic antioxidants could contribute to the preservation of TAC (Halliwell and Gutteridge, 2015), and the Nrf2 pathway may enhance the expression of multiple antioxidant enzymes (Kensler et al., 2007). Additionally, TAC assays have limitations in detecting dynamic changes in antioxidant status (Prior et al., 2005). The colonic mucosa may adapt to cadmium exposure through qualitative or quantitative modifications to antioxidants that are not reflected in total antioxidant power.

Inflammation is a regulated biological response, balancing protection and damage (Ahmed, 2011). The inflammatory marker TNF-α, observed in this study, is implicated in various inflammatory conditions (Aratani, 2018). TNF-α, a central cytokine in inflammation, recruits neutrophils and amplifies the immune response (Lejeune et al., 2023). This study found elevated TNF-α levels in cadmium-exposed rats across all age groups, highlighting cadmium's inflammatory effects on mucosal immune balance. Cadmium triggers TNF-α production through protein kinase C, p38 MAP kinase, and NF-kB signaling pathways in intestinal epithelial cells and macrophages (Kim et al., 2018). Cadmium-induced oxidative stress also prompts TNF-α release as a pro-inflammatory signal (Liu et al., 2009), and chronic exposure allows cadmium to accumulate in the colonic mucosa, sustaining TNF-α production (Pathak and Khandelwal, 2006).8-hydroxy-2-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, was significantly elevated in cadmium-treated old rats, indicating increased oxidative stress and mutagenesis with age. Aging cells accumulate DNA damage, leading to genomic instability,

which predisposes them to oxidative lesions (Moskalev et al., 2013). Old rats experience higher baseline DNA oxidation, which cadmium exacerbates. Cd-induced ROS attack DNA, generating oxidative lesions like 8-OHdG (Valko et al., 2005), and older organisms exhibit reduced DNA repair capacity, heightening susceptibility to cadmium's genotoxic effects (Moskalev et al., 2013). Cadmium disrupts DNA repair pathways, inhibiting enzymes involved in base excision repair (BER) and nucleotide excision repair (NER) (Hartwig, 2013). The aging gut epithelium also has increased permeability, worsened by cadmium exposure, which enhances bacterial translocation and inflammation (Man et al., 2014). Cadmium further disrupts the gut microbiome through oxidative stress and impaired antimicrobial defenses (Bhattacharyya et al., 2023). Histological analysis of cadmium-exposed rats reveals age-related differences in colonic damage. In young rats, mucosal ulceration (blue arrow) indicates acute epithelial disruption, reflecting cadmium's direct toxicity to the gastrointestinal barrier (Wang et al., 2021). Despite this, deeper layers, including the serous and muscular layers, remain intact, possibly due to the youthful tissue's regenerative capacity (Weiss, 2008). In middle-aged rats, the normal mucosal structure with submucosal enlargement (green arrow) suggests chronic inflammation or edema, potentially driven by cadmium-induced oxidative stress and immune cell infiltration (Salminen et al., 2012). This may reflect a shift from acute epithelial damage in younger rats to submucosal changes with age. In older rats, arteriolar dilation (white arrow) suggests impaired microcirculation, likely due to age-related vascular decline and increased oxidative stress from cadmium (Cuypers et al., 2010). The vascular abnormalities could result from ROS generation and reduced nitric oxide availability, leading to endothelial dysfunction (Unsalet al., 2020). These findings support the notion that cadmium's effects worsen with age, transitioning from mucosal injury to chronic vascular changes (Liu et al., 2014).

CONCLUSION

Exposure to cadmium exacerbates age related intestinal damage through impaired defense system and oxidative stress.This study revealed that cadmium exposure in old age female ratsinduces oxidative stress (increased MDA, 8-OHdG levels) leading to a decrease in antioxidants enzymes activities (SOD, GSH, Catalase, TAC)and body weight. Additionally, Cadmium exposure in old age female ratsinduces inflammation by elevating pro-inflammatory marker (TNF-alpha) and total bacterial count, and alters histological changes of the colon.These findings suggest that, old age females are more susceptible to cadmium toxicity due to age related decline in their antioxidant functions and immune system compared to the young and middle-age females.

Ethical Approval

The experimental procedure was approved by the Faculty of Basic Medical Sciences, LAUTECH Ethical Approval Committee.Ethical Research Committee Approval Number**:** ERCFBMSLAUTECH:051/06/2024.The animal handling procedure was done according to the guidelines for the use and care of laboratory animals, as recommended by the animal care and use research ethic committee of LAUTECH, were followed.

Disclaimer (Artificial intelligence)

We 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.

REFERENCES

Adegoke, A. G., Salami, A. T., & Olaleye, S. B. (2017). Cadmium exacerbates acetic acid induced experimental colitis in rats. *Eur Exp Biol*, *7*(5), 27.

Adegunlola JG, Afolabi OK, Akhigbe RE, Adegunlola GA, Adewumi OM, Oyeyipo IP, Afolabi AO. Lipid peroxidation in brain tissue following administration of low and high doses of arsenite and L-ascorbate in Wistar strain rats. Toxicology International. 2012;19(1):47.

Afolabi OA, Anyogu DC, Hamed MA, Odetayo AF, Adeyemi DH, Akhigbe RE. Glutamine prevents upregulation of NF-kB signaling and caspase 3 activation in ischaemia/reperfusion-induced testicular damage: An animal model. Biomedicine & Pharmacotherapy. (2022) Jun 1;150:113056.

Ahmed, M. (2011). Inflammation and cancer: A review. Journal of Advanced Pharmaceutical Technology & Research, 2(3), 174.

Al-Hada, N. M., Saion, E., Talib, Z. A., & Shaari, A. H. (2016). Cadmium suppresses catalase activity and expression in human lung cells. Toxicology in Vitro, 28(5), 767-775.

Ali S, Sunar S, Saha P, Mukherjee P, Saha S. & Dutta S (2021). Drinking water quality assessment of river Ganga in West Bengal, India through integrated statistical and GIS techniques. Water Sci Technol 84:2997–3017.

Andres, E., &Serraj, K. (2012). Anemia in elderly: An update. Journal of Blood Medicine, 3, 159-167.

Aratani, Y. (2018). Role of tumor necrosis factor-alpha in inflammation and disease. Journal of Inflammation Research, 11, 305-315.

Azzouz, L. L., & Sharma, S. (2018). Physiology, large intestine.

Bachmann, M. C., Bellalta, S., Basoalto, R., Gómez-Valenzuela, F., Jalil, Y., Lépez, M., ... & von Bernhardi, R. (2020). The challenge by multiple environmental and biological factors induce inflammation in aging: their role in the promotion of chronic disease. *Frontiers in Immunology*, *11*, 570083.

Benz, C. C. (2008). Onset of androgen deprivation therapy: What is the impact on risk factors for cardiovascular disease and diabetes?. Clinical Genitourinary Cancer, 6(2), 91-96.

Bernard, A. (2008). Cadmium & human health: From toxicity to essentiality. Journal of Applied Toxicology, 28(5), 608-611.

Bernhoft, R. A. (2013). Cadmium toxicity and treatment. The Scientific World Journal, 2013,1-7.

Besarab, A., Bolton, W. K., Browne, R., Egrie, J. C., Nissenson, A. R., Okamoto, D. M., ... & Van Wyck, D. B. (1999). The effects of normal as compared with low hematocrit values

in patients with cardiac disease who are receiving hemodialysis and epoetin. New England Journal of Medicine, 339(9), 584-590.

Bhattacharya, P., Banerjee, P., Hazra, S., Sengupta, S., & Dey, S. (2024). Lead, Cadmium, and Chromium Contamination in Soil–Plant System: Global Situation, Impact, and Dietary Risk Delineation. In *Environmental Contaminants* (pp. 47-79). Apple Academic Press.

Bhattacharyya, K., Sen, D., Laskar, P., Saha, T., Kundu, G., Ghosh Chaudhuri, A., & Ganguly, S. (2023). Pathophysiological effects of cadmium (II) on human health-a critical review. *Journal of Basic and Clinical Physiology and Pharmacology*, *34*(3), 249-261.

Casalino, E., Sblano, C., &Landriscina, C. (1997). Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. *Archives of biochemistry and biophysics*, *346*(2), 171-179.

Chakravorty, M., Nanda, M., Bisht, B., Sharma, R., Kumar, S., Mishra, A., ... & Kumar, V. (2023). Heavy metal tolerance in microalgae: detoxification mechanisms and applications. *Aquatic Toxicology*, *260*, 106555.

Chakravorty, S., Malvi, A., Chaturvedi, A., Sonkusare, K., & Dave, N. (2020). Glutathione–the master antioxidant. *International Journal of Medical Research and Pharmaceutical Sciences*, *7*(2), 1-11.

Chung, H. Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A. Y., ... &Leeuwenburgh, C. (2009). Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing research reviews*, *8*(1), 18-30.

Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., ... & Smeets, K. (2010). Cadmium stress: an oxidative challenge. *Biometals*, *23*, 927-940.

Duan, Y., Wang, Y., Huang, J., Li, H., Dong, H., & Zhang, J. (2021). Toxic effects of cadmium and lead exposure on intestinal histology, oxidative stress response, and microbial community of Pacific white shrimp Litopenaeusvannamei. *Marine Pollution Bulletin*, *167*, 112220.

Fiseha, M., Mohammed, M., Ebrahim, E., Demsiss, W., Tarekegn, M., Angelo, A., ... & Tsegaye, A. (2022). Common hematological parameters reference intervals for apparently healthy pregnant and non-pregnant women of South Wollo Zone, Amhara Regional State, Northeast Ethiopia. *Plos one*, *17*(7), e0270685.

Franco, M. C., Kawamoto, E. M., Gorjão, R., Rastelli, V. M., Curi, R., Scavone, C., ... & Sesso, R. (2007). Biomarkers of oxidative stress and antioxidant status in children born small for gestational age: evidence of lipid peroxidation. *Pediatric research*, *62*(2), 204-208.

Hada, H., Shiraki, T., Watanabe-Matsui, M., & Igarashi, K. (2014). Hemopexin-dependent heme uptake via endocytosis regulates the Bach1 transcription repressor and heme oxygenase gene activation. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1840*(7), 2351-2360.

Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine. Oxford University Press.

Hartwig, A. (2013). Cadmium and cancer: Mechanistic and epidemiological aspects. Journal of Applied Toxicology, 33(11), 1111-1124.

Horiguchi, H., Sato, M., Konno, N., & Fukushima, M. (1996). Long-term cadmium exposure induces anemia in rats through hypoinduction of erythropoietin in the kidneys. *Archives of toxicology*, *71*, 11-19.

Jacquillet, G., Barbier, O., Rubera, I., Tauc, M., Borderie, A., Namorado, M. C., ... &Cougnon, M. (2007). Cadmium causes delayed effects on renal function in the offspring of cadmium-contaminated pregnant female rats. *American Journal of Physiology-Renal Physiology*, *293*(5), F1450-F1460.

Johri, N., Jacquillet, G., & Unwin, R. (2010). Heavy metal poisoning: the effects of cadmium on the kidney. *Biometals*, *23*, 783-792.

Jokanović M. & Kosanović M. (2010). Neurotoxic effects in patients poisoned with organophosphorus pesticides. Environ ToxicolPharmacol 29:195–201

Kensler, T. W., Wakabayashi, N., & Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.*, *47*(1), 89-116.

Khalil, M., Iqbal, M., Turan, V., Tauqeer, M., Farhad, M., Ahmed, A. & Yasin, S. (2022). Environmental micropollutants, *62*(2), 201-232.

Khoubnasabjafari, M., Ansarin, K., &Jouyban, A. (2015). Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *BioImpacts: BI*, *5*(3), 123.

Khoubnasabjafari, M., Ansarin, K., &Jouyban, A. (2016). Salivary malondialdehyde as an oxidative stress biomarker in oral and systemic diseases. *Journal of dental research, dental clinics, dental prospects*, *10*(2), 71.

Kim, E. A., Kim, S. Y., Ye, B. R., Kim, J., Ko, S. C., Lee, W. W., ... & Heo, S. J. (2018). Anti-inflammatory effect of Apo-9′-fucoxanthinone via inhibition of MAPKs and NF-kB signaling pathway in LPS-stimulated RAW 264.7 macrophages and zebrafish model. *International Immunopharmacology*, *59*, 339-346.

Kumar, R., Jain, K., Beeke, C., Price, T. J., Townsend, A. R., Padbury, R., ... &Karapetis, C. S. (2013). A population-based study of metastatic colorectal cancer in individuals aged≥ 80 years: Findings from the South Australian Clinical Registry for Metastatic Colorectal Cancer. *Cancer*, *119*(4), 722-728.

Lamas, G. A., Bhatnagar, A., Jones, M. R., Mann, K. K., Nasir, K., Tellez-Plaza, M., ... & American Heart Association Council on Epidemiology and Prevention; Council on Cardiovascular and Stroke Nursing; Council on Lifestyle and Cardiometabolic Health; Council on Peripheral Vascular Disease; and Council on the Kidney in Cardiovascular Disease. (2023). Contaminant metals as cardiovascular risk factors: a scientific statement from the American Heart Association. *Journal of the American Heart Association*, *12*(13), e029852.

Lech T. &Sadlik J.K. (2017). Cadmium concentration in human autopsy tissues. Biol Trace Elem Res;179:172–177.

Lee, H. H., Owens, V. N., Park, S., Kim, J., & Hong, C. O. (2018). Adsorption and precipitation of cadmium affected by chemical form and addition rate of phosphate in soils having different levels of cadmium. *Chemosphere*, *206*, 369-375.

Lejeune, M., Reverté, L., Gallardo, N., Sauras, E., Bosch, R., Mata, D., ... & López, C. (2023). Matrix Metalloproteinase-9 Expression Is Associated with the Absence of Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer Patients. *International Journal of Molecular Sciences*, *24*(14), 11297.

Liu, C. M., Ma, J. Q., & Sun, Y. Z. (2011). Protective role of puerarin on lead-induced alterations of the hepatic glutathione antioxidant system and hyperlipidemia in rats. *Food and chemical toxicology*, *49*(12), 3119-3127.

Liu, Y., Li, Y., Liu, K., & Shen, J. (2014). Exposing to cadmium stress cause profound toxic effect on microbiota of the mice intestinal tract. *PloS one*, *9*(2), e85323.

Man, S. M., Hopkins, L. J., Nugent, E., Cox, S., Glück, I. M., Tourlomousis, P., ... & Bryant, C. E. (2014). Inflammasome activation causes dual recruitment of NLRC4 and NLRP3 to the same macromolecular complex. *Proceedings of the National Academy of Sciences*, *111*(20), 7403-7408.

Mangiola, F., Nicoletti, A., Gasbarrini, A., & Ponziani, F. R. (2018). Gut microbiota and aging. *European Review for Medical & Pharmacological Sciences*, *22*(21).

Men, H., Young, J.L., Zhou, W., Zhang, H., Wang, X., Xu, J., Lin, Q., Tan, Y., Zheng, Y. and Cai, L., 2021. Early-life exposure to low-dose cadmium accelerates diethylnitrosamine and diet-induced liver cancer. *Oxidative Medicine and Cellular Longevity*, *2021*.

Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972;247(10):3170-3175.

Moskalev, A. A., Shaposhnikov, M. V., Plyusnina, E. N., Zhavoronkov, A., Budovsky, A., Yanai, H., &Fraifeld, V. E. (2013). The role of DNA damage and repair in aging through the prism of Koch-like criteria. *Ageing research reviews*, *12*(2), 661-684.

Ognjanović, B. I., Marković, S. D., Pavlović, S. Z., Žikić, R. V., &Štajn, A. Š. (2008). Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. *Physiological research*, *57*(3).

Orisakwe OE, Igwilo IO, Afonne OJ, Maduabuchi UJ. Heavy metal hazards of Nigerian sachet water. Arch Environ Health. 2006;61:209–313.

Pathak, N., & Khandelwal, S. (2006). Influence of cadmium on murine thymocytes: potentiation of apoptosis and oxidative stress. *Toxicology letters*, *165*(2), 121-132.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, *53*(10), 4290-4302.

Rafati R.M. &Moghadamnia A.A. (2010). Organophosphorus compounds poisoning. J Babol Univ Med Sci.;12:71–85.

Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S. &Moghadamnia AA. (2014). Current approaches of the management of mercury poisoning: need of the hour. DARU.;22:46.

Renugadevi, J., & Prabu, S. M. (2010). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Experimental and Toxicologic Pathology*, *62*(2), 171-181.

Salminen, A., Kaarniranta, K., & Kauppinen, A. (2012). Inflammaging: disturbed interplay between autophagy and inflammasomes. *Aging (Albany NY)*, *4*(3), 166.

Salminen, A., Kaarniranta, K., & Kauppinen, A. (2013). Crosstalk between oxidative stress and SIRT1: impact on the aging process. *International journal of molecular sciences*, *14*(2), 3834-3859.

Satarug, S., Vesey, D. A., and Gobe, G. C. (2022).Mitigation of Cadmium Toxicity through Modulation of the Frontline Cellular Stress Response. *Stresses*, *2*(3), 355-372.

Silvestrini, A., Meucci, E., Ricerca, B. M., & Mancini, A. (2023). Total antioxidant capacity: biochemical aspects and clinical significance. *International Journal of Molecular Sciences*, *24*(13), 10978.

Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry. 1972;47(2):389-394.

Sinicropi, M.S.; Amantea, D.; Caruso, A.; Saturnino, C. Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning. Arch. Toxicol. 2010, 84, 501–520.

Tang, Q., Jin, G., Wang, G., Liu, T., Liu, X., Wang, B., & Cao, H. (2020). Current sampling methods for gut microbiota: a call for more precise devices. *Frontiers in cellular and infection microbiology*, *10*, 151.

Teskey, G., Abrahem, R., Cao, R., Gyurjian, K., Islamoglu, H., Lucero, M., ... &Venketaraman, V. (2018). Glutathione as a marker for human disease. *Advances in clinical chemistry*, *87*, 141-159.

Tinkov, A. A., Gritsenko, V. A., Skalnaya, M. G., Cherkasov, S. V., Aaseth, J., &Skalny, A. V. (2018). Gut as a target for cadmium toxicity. *Environmental Pollution*, *235*, 429-434.

Unsal, V., Dalkıran, T., Çiçek, M., &Kölükçü, E. (2020). The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. *Advanced pharmaceutical bulletin*, *10*(2), 184.

Valko, M. M. H. C. M., Morris, H., & Cronin, M. T. D. (2005). Metals, toxicity and oxidative stress. *Current medicinal chemistry*, *12*(10), 1161-1208.

Wang, Z., Sun, Y., Yao, W., Ba, Q., & Wang, H. (2021). Effects of cadmium exposure on the immune system and immunoregulation. *Frontiers in immunology*, *12*, 695484.

Weiss, B. (Ed.). (2012). *Aging and vulnerability to environmental chemicals: age-related disorders and their origins in environmental exposures* (Vol. 16). Royal Society of Chemistry.

Weldeslassie T, Naz H, Singh B, Oves M (2018) Chemical contaminants for soil, air and aquatic ecosystem. In: Oves M, Zain Khan M, M.I. Ismail I (eds) Modern age environmental problems and their remediation. Springer International Publishing, Cham, pp 1–22.

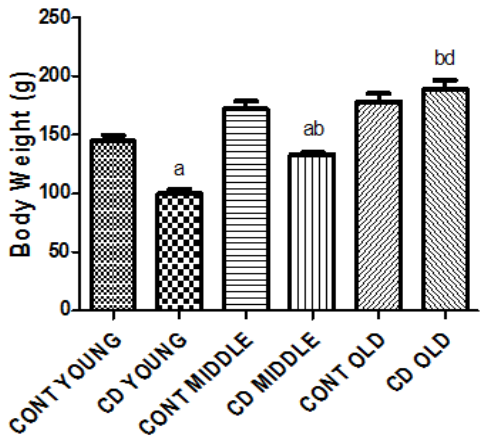
Xie, X., He, Z., Chen, N., Tang, Z., Wang, Q., & Cai, Y. (2019). The roles of environmental factors in regulation of oxidative stress in plant. *BioMed research international*, *2019*(1), 9732325.

Yarborough, A., Zhang, Y. J., Hsu, T. M., & Santella, R. M. (1996). Immunoperoxidase detection of 8-hydroxydeoxyguanosine in aflatoxin B1-treated rat liver and human oral mucosal cells. *Cancer Research*, *56*(4), 683-688.

Zhou, S, (2013). Mutation Research/Reviews in Mutation Research, 752(1), 1-13.

Zinellu, A., &Mangoni, A. A. (2021). A systematic review and meta-analysis of the effect of statins on glutathione peroxidase, superoxide dismutase, and catalase. *Antioxidants*, *10*(11), 1841.

GRAPHS



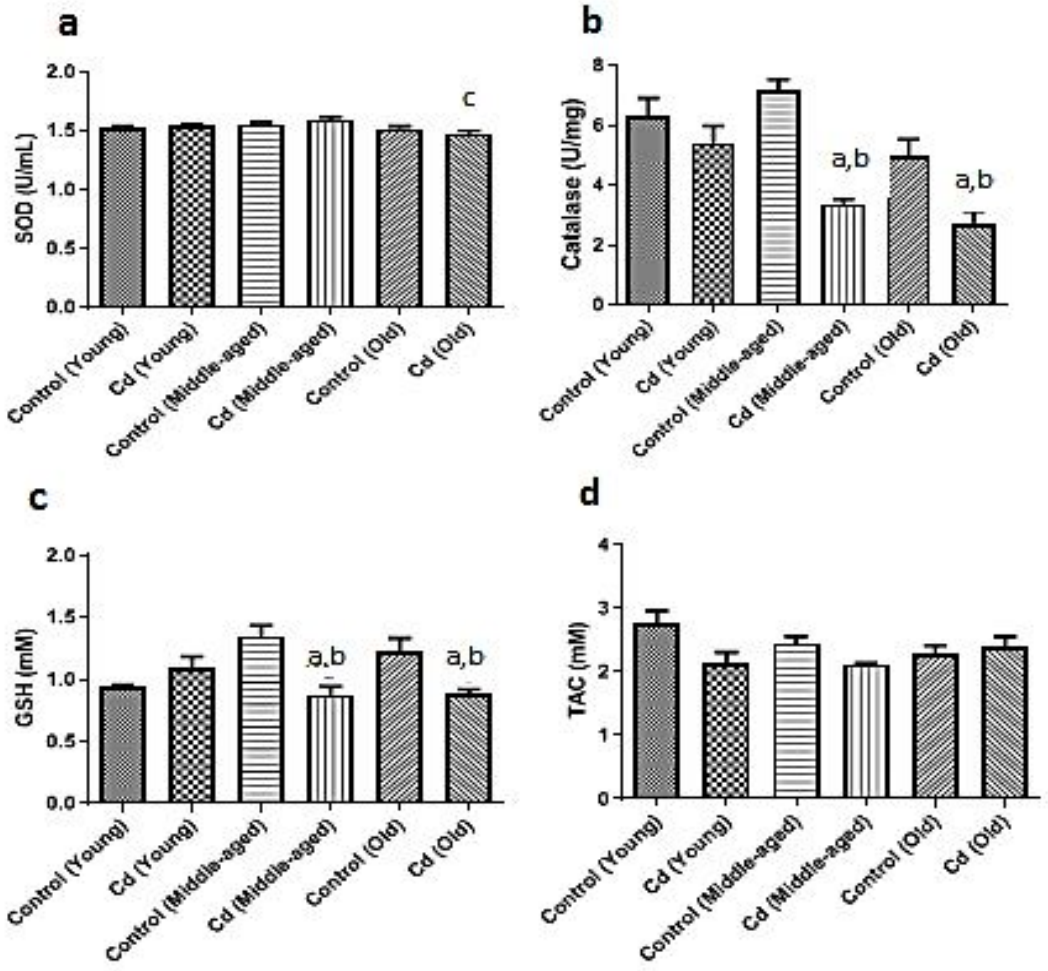


Figure 1: Effect of age on colonic antioxidants activities superoxide dismutase[a], catalase[b], glutathione[c], and total antioxidant capacity[d] of cadmium exposed female Wistar rats.

a - Represents significance at (P<0.05) comparing the three Cd groups with their controls.

b - Represents significant difference (P<0.001) compared to Cd (young) group.

c - Represents significant difference (P<0.01) compared to Cd (middle-age) group.

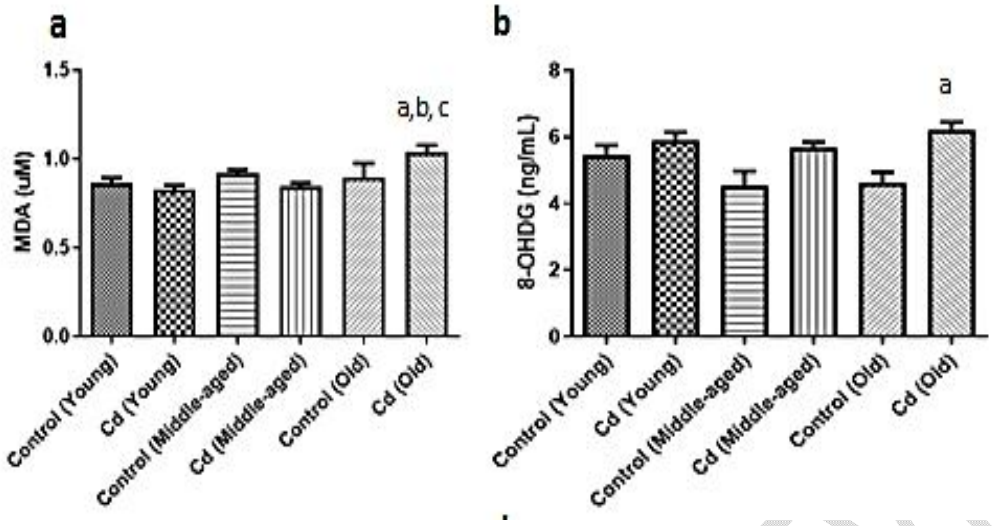


Figure 2: Effect of age on colonic Malondialdehyde concentration[a] and 8-hydroxy-2-deoxyguanosineactivity[b] of cadmium exposed female Wistar rats.

a - Represents significance at (P<0.05, 0.01) comparing the three Cd groups with their controls.

b - Represents significant difference (P<0.05) compared to Cd (young) group.

c - Represents significant difference (P<0.05) compared to Cd (middle-age) group.

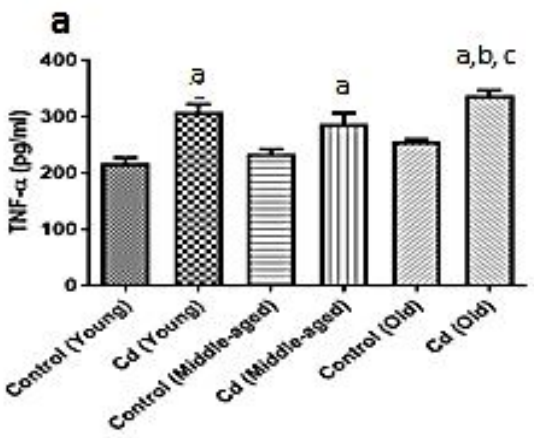


Figure 3: Effect of age on colonic tumor necrosis factor–alpha of cadmium exposed female Wistar rats.

a - Represents significance at (P<0.05) comparing the three Cd groups with their controls.

b - Represents significant difference (P<0.05) compared to Cd (young) group.

c - Represents significant difference (P<0.05) compared to Cd (middle-age) group.

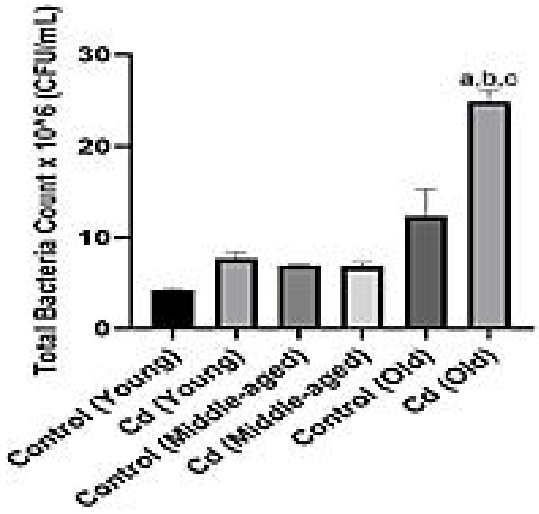
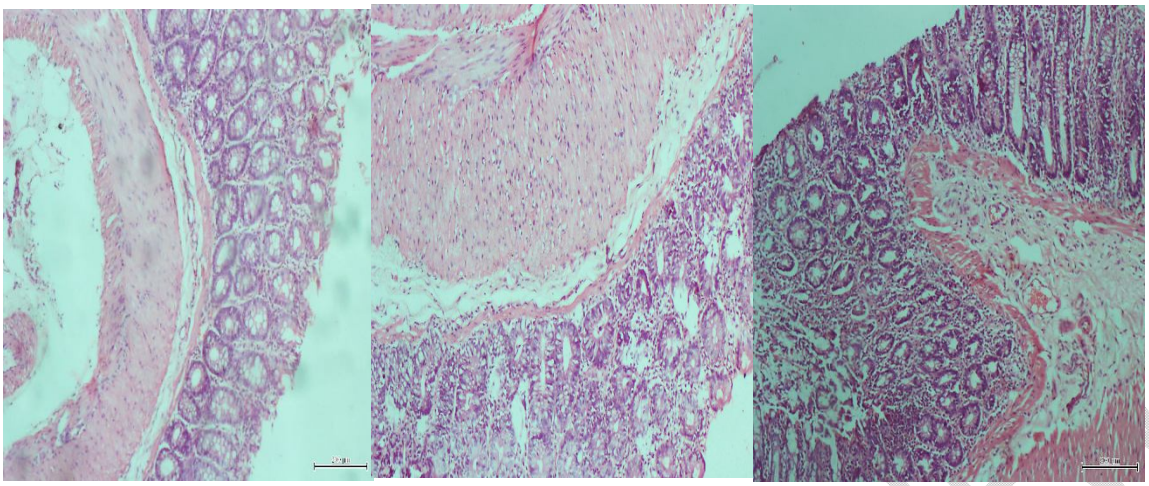


Figure 4: Effect of age on total colonic bacterial count alpha of cadmium exposed female Wistar rats.

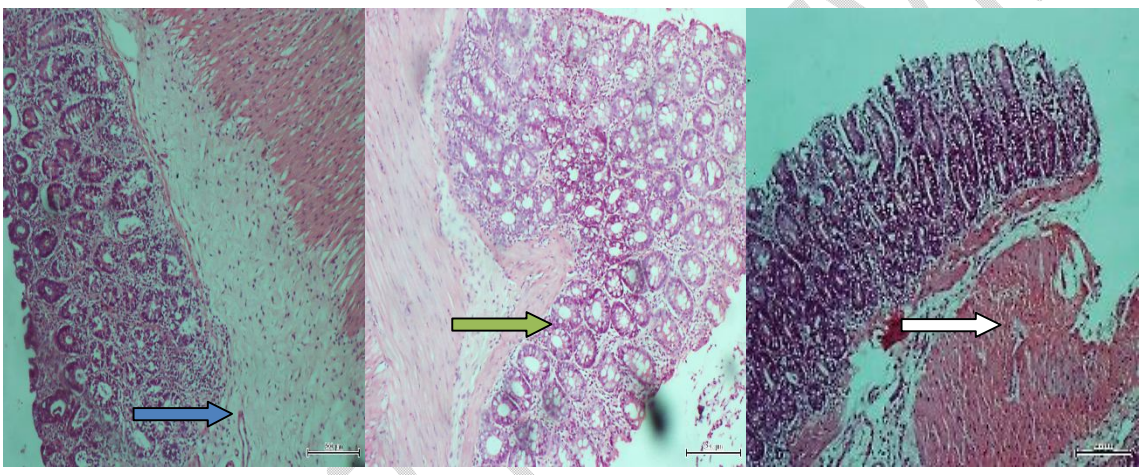
a - Represents significance at (P<0.05, 0.001) comparing the three Cd groups with their controls.

b - Represents significant difference (P<0.001) compared to Cd (young) group.

c - Represents significant difference (P<0.001) compared to Cd (middle-age) group.



Control (young age) Control (middle age) Control (Old age)



Cadmium (young age) Cadmium (middle age) Cadmium (Old age)

Figure 5: Photomicrographs of the structure of wall of the colon of cadmium-exposed female Wistar rats (H&E ×100)

Mucosal Ulceration
Submucosal Enlargement
Arteriolar Dilation