International Research Journal of Gastroenterology and Hepatology

Volume 7, Issue 1, Page 105-117, 2024; Article no.IRJGH.124280

Effect of Age on Colonic Damage Induced by Cadmium Exposure in Female Wistar Rats

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Authors' contributions

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

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124280

Original Research Article

Received: 01/08/2024
Accepted: 04/10/2024
Published: 31/10/2024

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 age affects colonic damage caused by cadmium exposure in female Wistar rats.

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Cite as: *Ige, Serah Funke, Ibukunoluwa Victor Fayemi, Julius Ayomide Ayoola, Mariam Ololade Olasupo, Olajumoke Deborah Ogunleye, Gbenga Opeyemi Owolabi, and Taiwo Adebayo Ajagbe. 2024. "Effect of Age on Colonic Damage Induced by Cadmium Exposure in Female Wistar Rats". International Research Journal of Gastroenterology and Hepatology 7 (1):105-17. https://journalirjgh.com/index.php/IRJGH/article/view/103.*

Methods: 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. Data were analyzed using One Way Analysis of Variance (ANOVA) 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 result shows a significant reduction in the body weight of Cd exposed young and middle-age groups only compared with their respective control groups (P=0.001; 0.05). There was no significant difference in SOD activity among Cd exposed 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 Cd exposed old-age groups (P=0.01; 0.05) compared to their controls and Cd exposed young age group. MDA and 8-OHDG levels increased significantly in the Cd exposed old-age group (P=0.01) compared to its control. TNF-α and total colonic bacterial count were significantly increased in the Cd exposed old-age group compared to other Cd exposed groups (P=0.001; 0.001). Histological examination showed different tissue damage patterns in the colon across Cd-exposed age groups, with more severe changes in older rats.

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

Keywords: Wistar rats; cadmium; heavy metal; animal models.

1. 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 [1]. Exposure to cadmium mainly occurs through ingestion of contaminated food and water, inhalation of polluted air, and sometimes through skin absorption [2]. 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 [3]. Over the past few decades, the use of chemicals both in industries and local settings has dramatically increased, resulting in heightened chemical exposure risks [4, 5]. Industrial chemicals are commonly found in products such as soaps, cosmetics, plastics, inks, cleaning agents, and other everyday materials used in homes and workplaces [6]. 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 [7].

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 [8]. 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 [9]. The gastrointestinal (GI) tract, particularly the intestines, is highly susceptible to cadmium toxicity [10]. 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 [11]. Additionally, cadmium negatively affects gut microflora, which can further impact intestinal health [12]. Besides direct cytotoxicity, cadmium also promotes carcinogenesis through the production of reactive oxygen species (ROS), which is a primary driver of inflammation [13]. Elevated ROS levels can lead to oxidative stress and DNA damage [14]. Cadmium exposure is also linked to the assembly of the protein P53, which induces apoptosis in intestinal epithelial cells [13].

The colon, a key part of the GI tract responsible for nutrient absorption and waste elimination, is particularly vulnerable to cadmium's harmful effects [15]. 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 [16]. 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 [17]. 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 [18]. 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 [19]. The aging process, with its associated changes in gut structure, barrier integrity, and immune responses, could potentially exacerbate these effects [18].

2. MATERIALS AND METHODS

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 (purchased from 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. Cadmium chloride solution was prepared by dissolving 50 mg of $CdCl_2$ in 250 ml of distilled water. Sucrose solution was prepared by diluting 25 g of sucrose in 25 ml 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 100 ml of formalin in 900 ml of distilled water.

2.3 Study Design

Forty eight female Wistar rats were allotted into six groups of eight 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 ml of 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 [20].

2.4 Tissue 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.

2.5 Biochemical Analysis

Distal portion of colon in 0.25M sucrose solution was homogenized and centrifuged at 3000 rpm for 15 minutes. Colon (distal portion) homogenates were 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 Fridovich, [21] and Sinha et al., [22]. Glutathione (GSH) concentration was determined using the method of Sezgintürk & Dinçkaya, [23]. Total antioxidant capacity (TAC) and total protein levels were were assessed using Enzyme-Linked Immunosorbent Assay (ELISA) kit (Elabscience Biotechnology Inc. U.S.A.) following the manufacturer's instructions as previously described [24]. The content of MDA in the homogenates was determined spectrophotometrically using the method of Mateos et al., [25]. Oxidative DNA damage was measured by quantitative immunohistochemistry using a monoclonal antibody to 8-OHdG as described by Yarborough et al. [26]. TNF-α was assessed using enzyme-linked immunosorbent assay (ELISA) kits, as instructed by the manufacturer (eBIOSCIENCE, Bender MedSystems GmbH, Wien, Austria). Histological studies were carried out as described by Ogihara and Okabe [27]. The slides were observed and photomicrographs were taken at 100X magnification.

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 exposed young and middle-age groups only compared with their respective control groups (P=0.001; 0.05).

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 exposed groups compared to their controls. However, significant reduction in superoxide dismutase activity was only seen in Cd exposed old group compared to Cd exposed middle-age group (P= 0.001).

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

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

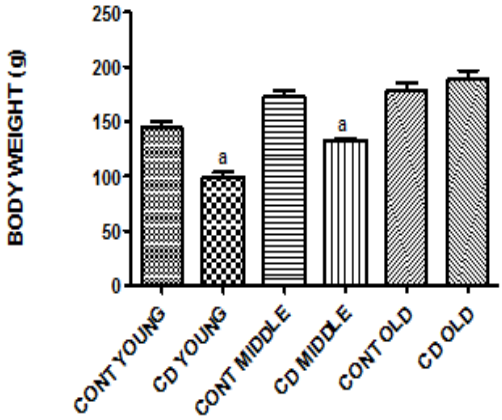


Fig. 1. Effect of age on body weight of cadmium exposed female Wistar rats
a - *Represents significance at (P<0.001; 0.05) comparing Cd exposed groups with their controls*

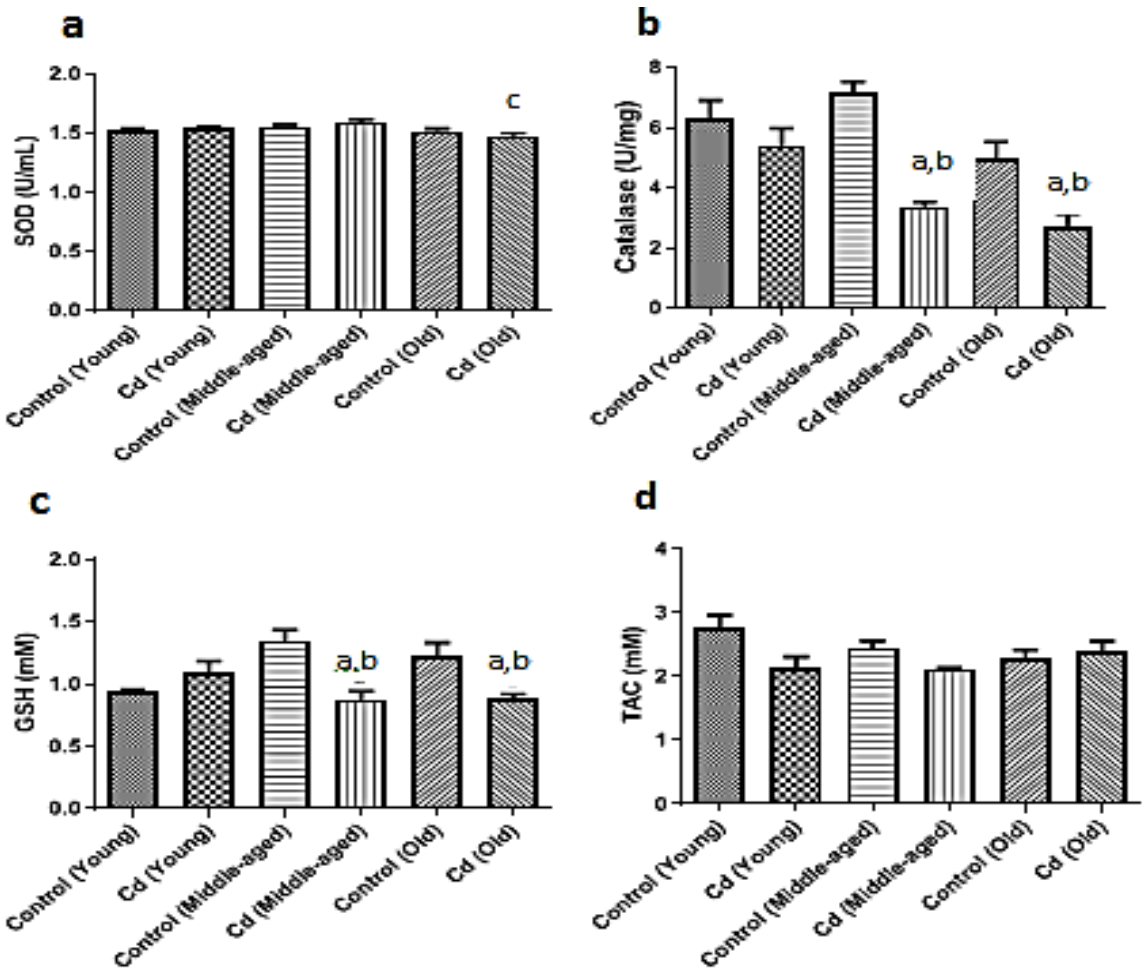


Fig. 2. Effect of age on colonic antioxidants activities superoxide dismutase[a], catalase[b], glutathione[c], and total antioxidant capacity[d] of cadmium exposed female Wistar rats
a - Represents significance at (P<0.05) comparing Cd exposed groups with their respective controls
b - Represents significant difference (P<0.05) compared to Cd exposed young group
c - Represents significant difference (P<0.05) compared to Cd exposed middle-age group

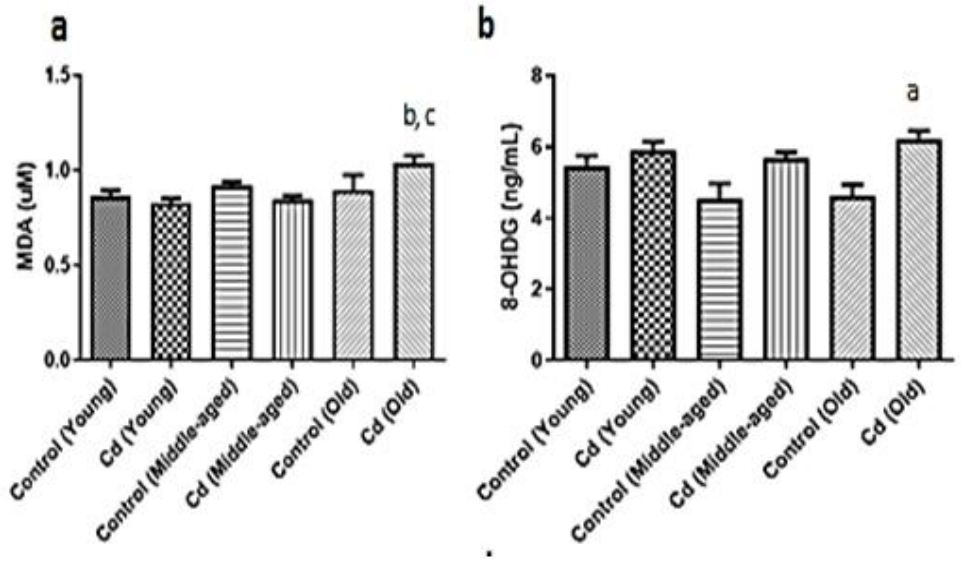


Fig. 3. Effect of age on colonic Malondialdehyde concentration[a] and 8-hydroxy-2-deoxyguanosine activity[b] of cadmium exposed female Wistar rats

a - Represents significance at (P<0.05) comparing Cd exposed old-age *group with its control*
b - Represents significant difference (P<0.05) compared to Cd exposed young group
c - Represents significant difference (P<0.05) compared to Cd exposed middle-age group

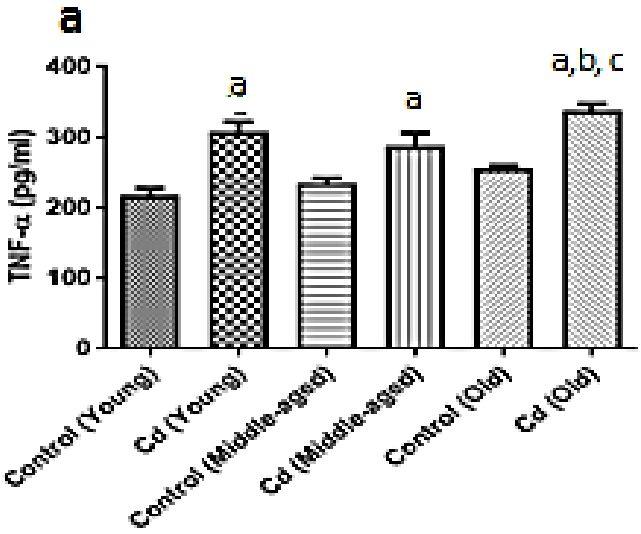


Fig. 4. Effect of age on colonic tumor necrosis factor–alpha of cadmium exposed female Wistar rats

a - Represents significance at (P<0.05) comparing Cd exposed groups with their controls
b - Represents significant difference (P<0.05) compared to Cd exposed young group
c - Represents significant difference (P<0.05) compared to Cd exposed middle-age group

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 exposed old groups compared to Cd exposed young and middle-age groups. (P=0.01; 0.05). No significant difference was seen in malondialdehyde (MDA) level Cd exposed young and middle-age groups compared to their respective control groups.

Also, a significant increase in 8-OHDG activity was seen only in Cd exposed old group compared to its control.

3.4 Effect of Age on Colonic Inflammatory Marker (TNF-α Concentration) of Cadmium Exposed Female Rats

There was a significant increase in TNF-α of Cd exposed groups compared to their control groups (P= 0.001). Additionally, TNF-α significant increase in Cd exposed old age groups compared to Cd exposed 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 exposed 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 exposed 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 exposed young rats showed ulceration of the mucosa (blue arrow) with normal serous, muscular, and submucus layers compared to its control, Cadmium exposed middle-age rats showed normal serous, muscular, and mucus layers with submucosal enlargement (green arrow) compared to its control, and Cadmium exposed old-age rats showed normal serous, muscular, submucus and mucus layers with arteriolar dilation (white arrow) compared to its control.

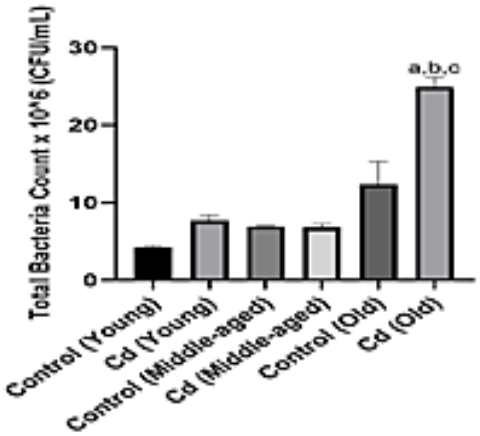
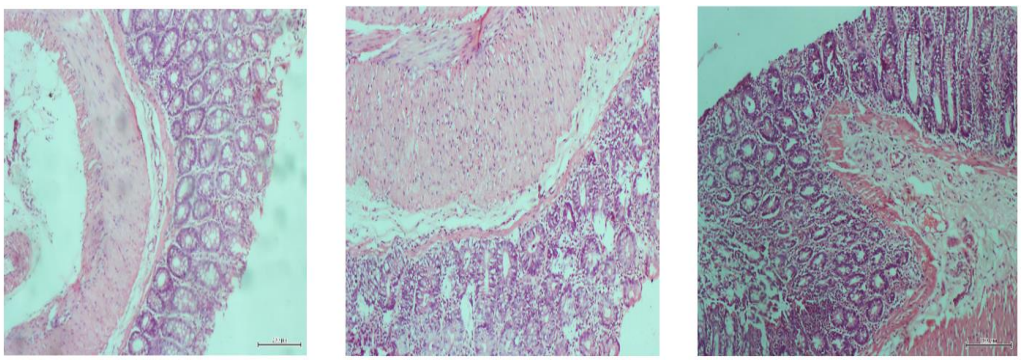


Fig. 5. Effect of age on total colonic bacterial count alpha of cadmium exposed female Wistar rats

a - Represents significance at (P<0.05) comparing Cd exposed old age group with its control
b - Represents significant difference (P<0.05) compared to Cd exposed young group
c - Represents significant difference (P<0.05) compared to Cd exposed middle-age group



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

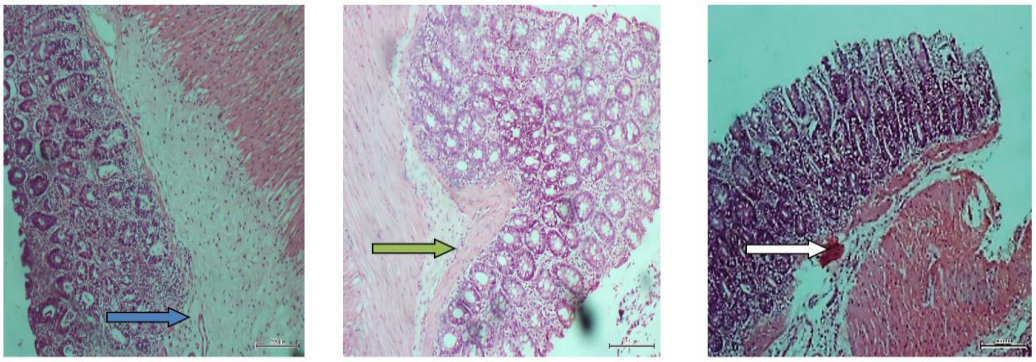


Fig. 6. Photomicrographs of the structure of wall of the colon of cadmium-exposed female Wistar rats (H&E ×100)

Mucosal Ulceration
Submucosal Enlargement
Arteriolar Dilation

4. DISCUSSION

Cadmium is a widespread environmental pollutant linked to gastrointestinal dysfunction in both animal models and humans [2]. Older individuals are particularly vulnerable to cadmium's adverse effects due to age-related physiological changes that influence metal absorption and toxicity [28]. 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 in weight in this study weighing, approximately 76.6% less than their controls, which may be a result of severe gastrointestinal dysfunction, characterized by reduced food intake, and impaired nutrient absorption [29]. Moreover, early-life exposure may disrupt critical growth signals, further explaining the stark differences in weight gain [30]. Middle-aged also exhibit suppressed weight gain when exposed to cadmium, although to a lesser extent, suggesting that metabolic toxicity persists throughout life [31]. 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, when compared to middle age group but not in middle-aged or young rats. There were significant reductions in colonic catalase levels with cadmium exposure in both middle-aged and older 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 [32]. 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 [32]. The observed decline of these antioxidants in Cd-exposed older and middle-aged rats reflects a weakened antioxidant defense system, suggesting disrupted redox homeostasis in the aging colon inic 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 [33], reducing the enzymes' catalytic activities. Age-related nutritional deficiencies may exacerbate cadmium's inhibitory effects on catalase function in older rats. Additionally, oxidative damage accumulates over time, and chronic inflammation in aging tissues can impair antioxidant activity [34].

Cadmium can also influence the gene expression and transcription factors responsible for antioxidant defense. Studies have shown that cadmium exposure can suppress catalase and other antioxidant enzymes by interfering with transcriptional regulation [35]. The significant reduction in catalase activity in Cd-exposed middle-aged rats and old compared to their

controls may be due to cadmium-induced inhibit catalase gene expression, compromising its synthesis and function [36]. Glutathione, known as a vital molecule in cells for defending cells against harmful molecules, detoxifying substances, maintaining redox balance, and regenerating antioxidants like vitamins C and E [37;38]. Glutathione (GSH) also neutralizes ROS, aids toxin excretion, enhances immune cell function, and indicates cellular health through its GSH/GSSG ratio [38]. Normally, GSH levels increase from young to middle age, boosting antioxidant defenses in response to age-related oxidative stress, with production peaking around maturity [39]. However, cadmium exposure significantly decreased GSH in middle-aged and older rats compared to their controls. Cadmium likely binds to and oxidizes GSH, either directly or by generating ROS [33]. Additionally, cadmium may inhibit GSH synthesis or deplete cofactors like selenium [40]. The marked decline in colonic GSH in Cd-exposed older rats suggests vulnerability linked to aging, as factors like reduced GSH synthesis enzymes activity with age, chronic inflammation, and mitochondrial dysfunction exacerbate cadmium-induced oxidative damage [41; 42].

Malondialdehyde (MDA) levels, a marker of lipid peroxidation, significantly increased in Cd-exposed older rats compared to young and middle-aged rats. MDA is a well-established biomarker for oxidative stress [43]. Aging is associated with increased production of ROS and reduced antioxidant defenses, leading to elevated oxidative stress [44]. Older rats likely experience more oxidative damage at baseline, which cadmium exposure exacerbates [45]. Impaired mitochondrial function in aging also makes older 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 [46]. 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 [47]. Non-enzymatic antioxidants could contribute to the preservation of TAC [48], and the Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) pathway may enhance the expression of multiple antioxidant enzymes [49]. Additionally, TAC assays have limitations in detecting dynamic changes in antioxidant status [50]. 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 [51]. The inflammatory marker TNF-α, observed in this study, is implicated in various inflammatory conditions [52]. TNF-α, a central cytokine in inflammation, recruits neutrophils and amplifies the immune response [53]. 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 [54]. Cadmium-induced oxidative stress also prompts TNF-α release as a pro-inflammatory signal [55], and chronic exposure allows cadmium to accumulate in the colonic mucosa, sustaining TNF-α production [56]. 8-hydroxy-2-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, was significantly elevated in cadmium-treated older rats, indicating increased oxidative stress and mutagenesis with age. Aging cells accumulate DNA damage, leading to genomic instability, which predisposes them to oxidative lesions [57]. Older rats experience higher baseline DNA oxidation, which cadmium induced. Cd-induced ROS attack DNA, generating oxidative lesions like 8-OHdG [58], and older rats exhibit reduced DNA repair capacity, heightening susceptibility to cadmium's genotoxic effects [57]. Cadmium disrupts DNA repair pathways, inhibiting enzymes involved in base excision repair (BER) and nucleotide excision repair (NER) [59]. The aging gut epithelium also has increased permeability, worsened by cadmium exposure, which enhances bacterial translocation and inflammation [60]. Cadmium further disrupts the gut microbiome through oxidative stress and impaired antimicrobial defenses [61]. Histological examination of cadmium-exposed rats reveals age-related differences in colonic damage. The results showed that Cd exposure had different effects on the Cd exposed groups: mucosal ulceration in Cd exposed young group, submucosal enlargement in Cd middle-age group, and arteriolar dilation in Cd exposed old group. In young rats, mucosal ulceration indicates acute epithelial disruption, reflecting

cadmium's direct toxicity to the gastrointestinal barrier [2]. Despite this, deeper layers, including the serous and muscular layers, remain intact, possibly due to the youthful tissue's regenerative capacity [62]. In middle-age rats, the normal mucosal structure with submucosal enlargement suggests chronic inflammation or edema, potentially driven by cadmium-induced oxidative stress and immune cell infiltration [34]. This may reflect a shift from acute epithelial damage in younger rats to submucosal changes with age. In older rats, arteriolar dilation suggests impaired microcirculation, likely due to age-related vascular decline and increased oxidative stress from cadmium [42]. The vascular abnormalities could result from ROS generation and reduced nitric oxide availability, leading to endothelial dysfunction [63]. These findings support the notion that cadmium's effects worsen with age, transitioning from mucosal injury to chronic vascular changes [64].

5. 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 rats induces oxidative stress (increased MDA, 8-OHdG levels) due to a decrease in antioxidants enzymes activities (SOD, GSH, Catalase, TAC). Additionally, Cadmium exposure in old age female rats induces inflammation by elevating pro-inflammatory marker (TNF-alpha) and total bacterial count, and alters histological structure 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 female rats.

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.

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.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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