

RESEARCH ARTICLE

OPEN ACCESS

Attenuation of cadmium chloride-induced oxidative damage in the testes of adult male Wistar rats administered with extracts of *Rauvolfia vomitoria* and *Aframomum melegueta*

Moronkeji A¹[ID](#), Adeniyi TD²[ID](#), Kehinde AO³[ID](#), Akinpelumi OM⁴[ID](#), Kayode I⁴[ID](#)

¹Department of Medical Laboratory Science, Faculty of Basic Medical Science, College of Health Sciences, Osun State University, Nigeria

²Department of Medical Laboratory Science, University of Ilorin, Kwara State, Nigeria

³Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Science, Achievers University, Owo, Ondo State, Nigeria

⁴Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Medical Sciences, Ondo, Nigeria

Submitted: 3rd August 2025

Accepted: 10th April 2026

Published: 30th September 2026

[ID](#): Orcid ID

Abstract

Objective: Cadmium is a testicular toxicant widely distributed in organs and tissues. It reduces sperm count, motility, and antioxidant enzyme levels, leading to alterations in testicular histology. This study investigated the protective effects of methanol extracts of *Aframomum melegueta* and *Rauvolfia vomitoria* against cadmium chloride (CdCl₂)-induced testicular toxicity in adult male Wistar rats, with specific focus on histopathological alterations, Johnsen's score, and myeloperoxidase (MPO) expression.

Method: Thirty-five adult male Wistar rats weighing an average of 200g were randomly assigned to five groups of seven rats each. The negative control was administered with distilled water, while the positive control group received 30mg/kg/BW of CdCl₂, groups 3 and 4 received 30mg/kg/BW of CdCl₂ and were treated with *A. melegueta*, *R. vomitoria* (200mg/kg/BW), respectively. Group 5 received both plant extracts. Cadmium was administered weekly for 21 days; extracts were orally administered once daily throughout the study.

Result: Histological evaluation indicated that treatment with leaf extracts of *R. vomitoria* or *A. melegueta* attenuated the CdCl₂-induced testicular toxicity, as demonstrated by a significantly higher Johnsen score compared with the CdCl₂-exposed untreated rats. Furthermore, co-administration of both extracts resulted in greater suppression of MPO levels than treatment with either plant extract alone, indicating a superior protective effect.

Conclusion: Histological assessment of the testes in treated rats showed a mitigating effect on CdCl₂-induced testicular damage, with co-administrative treatment of both plant extracts significantly attenuating CdCl₂-induced testicular toxicity, as evidenced by improved Johnsen's scores and reduced testicular MPO expression levels.

Keywords: Environmental pollutants, herbal extract, Johnsen's score, myeloperoxidase, Wistar rats

Plain English Summary

Cadmium is a toxic heavy metal released through industrial activity that accumulates in the body and damages the testes, impairing sperm production and contributing to male infertility. This study investigated

Correspondence:

Kehinde Adedapo

Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Science

Achievers University, Owo

Ondo State, Nigeria

+2347033935591, Kehindeadedapo@achievers.edu.ng

whether leaf extracts of two African medicinal plants; *Aframomum melegueta* and *Rauvolfia vomitoria*, could protect against this damage in male rats. Thirty-five rats were divided into five groups: a healthy control, a cadmium-exposed untreated group, and three groups that received cadmium alongside either *A. melegueta*, *R. vomitoria*, or both extracts combined. After 21 days, the testes were examined under a microscope and sperm-producing activity was graded using Johnsen's scoring system. Levels of myeloperoxidase (MPO), an enzyme that indicates inflammation and tissue injury, were also measured. Results showed that cadmium caused significant structural damage to testicular tissue and disrupted sperm production. Both plant extracts partially reversed this damage, with *R. vomitoria* showing a superior protective effect when used alone. Notably, the combination of both extracts produced the greatest reduction in testicular MPO levels, suggesting a stronger anti-inflammatory effect when used together. These findings indicate that *A. melegueta* and *R. vomitoria* hold promise as natural protective agents against cadmium-induced testicular toxicity, though further studies involving longer treatment periods and human trials are needed to confirm their therapeutic potential.

Introduction

Environmental contamination has recently emerged as a major global issue, driven by increasing urbanisation and industrialisation, which contribute to the release of harmful pollutants such as chemicals, pesticides, petroleum products, and heavy metals into water, soil, and air, negatively affecting both plants and humans (1, 2). Heavy metals are significant environmental pollutants due to their toxicity, long-lasting presence in the atmosphere, and bioaccumulation in the human body. Most heavy metals occur naturally, but some are anthropogenic. They are characterised by their high atomic mass and toxicity to living organisms (3). Cadmium (Cd), discovered by Friedrich Stromeyer in 1817, is a soft, silvery-white metal found in zinc ore. It comprises 0.00005% of Earth's crust and spreads easily through air, water, and soil, posing health risks via the plant-to-human pathway (4, 5). Cadmium accumulates in organs like the liver, heart, and testes, leading to severe health issues and death. It is a group 12 element with atomic number 48 and configuration $[Kr] 4d^{10}5s^2$ (1, 6). Cadmium is considered one of the most hazardous heavy metals due to industrial discharge and its long biological half-life (20–40 years) (7, 8, 9). It accumulates in the body and is classified as carcinogenic by the IARC (10). Environmental exposure to cadmium contributes to male infertility by affecting reproductive development, with infertility affecting 10–15% of couples globally (11, 12). Oxidative stress causes the recruitment of inflammatory cells and the creation of proinflammatory cytokines, which results in the initial stage of inflammation (13). In the male reproductive system, once there is irregularity or an increase in the production of reactive oxygen species (ROS), it damages the fluidity of the sperm plasma membrane, which affects the integrity of DNA in the sperm nucleus, leading to spermatogenesis dysfunction, toxic effects on sperm, and causing lipid peroxidation

(14). Cadmium exposure disrupts spermatogenesis, lowers sperm quality, and contributes to infertility by inducing oxidative stress, testicular inflammation, and cell apoptosis (15, 16). It impairs androgenic function, damages the blood-testis barrier, and alters epigenetic regulation (17). Histologically, it reduces tunica albuginea thickness, tubular lumen diameter, and interstitial space while increasing epithelial height and seminiferous tubule diameter (18). Herbal remedies, used since the dawn of humanity, are increasingly becoming a cornerstone of modern healthcare. They continue to be widely utilised and play a significant role in global trade (19, 20). Due to the high cost of conventional therapies and surgical procedures, medicinal plants recognised for their therapeutic value and safety in both modern and orthodox medicine have been widely used in the treatment of male sexual disorders (15). *Rauvolfia vomitoria* is a perennial shrub of the Apocynaceae family, native to tropical regions of Africa, South America, and Asia. In Nigeria, it is known by various ethnic names such as "Asofeyeje" (Yoruba) and "Ira" (Igbo) (21). Traditionally, it is used to enhance male sexual and reproductive function and to treat ailments like jaundice and gastrointestinal issues in children (22). Its pharmacological applications include antihypertensive and sedative effects. The plant contains active phytochemicals such as alkaloids (e.g., reserpine, serpentinine), saponins, and steroids like ergosterol. Alkaloids and saponins (23) are linked to libido enhancement and aphrodisiac activity, making *R. vomitoria* a significant herbal remedy for male sexual health (24). *Aframomum melegueta* (Alligator pepper), a tropical herb of the Zingiberaceae family, is widely used for its medicinal and nutritional benefits (25). Known by names like Grains of Paradise and Oseioji (26), it possesses antibacterial, anticancer, hepatoprotective, and antidiabetic properties (27). These effects are attributed to its rich

phytochemical profile, including essential oils, flavonoids, alkaloids, terpenoids, vitamins, and minerals (28). Myeloperoxidase (MPO) is a lysosomal peroxidase enzyme predominantly released by activated neutrophils, exhibiting potent pro-oxidative, proinflammatory, and antibacterial activities (29, 30). Notably, elevated MPO levels in seminal fluids have been correlated with decreased sperm count and motility in healthy young men (31). While previous studies have established the role of myeloperoxidase in impairing sperm quality and highlighted the antioxidative and immunomodulatory effects of *A. melegueta*, as well as its combined influence with *R. vomitoria* on MPO and NF- κ B expression in murine models, there remains a paucity of data specifically addressing the comparative testicular protective effects of these extracts in cadmium-induced toxicity (31, 32, 33, 34). Furthermore, the mechanistic link between MPO modulation and spermatogenic restoration, particularly as assessed by histological indices such as Johnsen's score, has not been comprehensively explored in this context. This study evaluates the protective effects of *A. melegueta* and *R. vomitoria* on reproductive histology and the modulatory role on myeloperoxidase expression in the testes of cadmium-exposed experimental rats.

Materials and Methods

Procurement of Chemicals

Cadmium chloride hemihydrate ($\text{CaCl}_2 \cdot 2.5\text{H}_2\text{O}$ = 228.34) with batch number 6802/3 and product number CO262 was a product of Surechem Products Limited (United Kingdom).

Plant Collection, Extraction, and Preparation of Extracts

The leaves of *R. vomitoria* and *A. melegueta* were obtained from a farm in Laje Village, Ondo City, Ondo State, Nigeria. The leaves were then identified by a professional in the Plant Biology and Biotechnology department of the University of Medical Sciences and were assigned herbarium numbers 0059 and 0069, respectively. The leaves were thoroughly cleaned to remove dirt, then shade-dried for one week. After drying, they were ground into a fine powder using an electric blender. The powdered leaves were soaked in 75–95% methanol and extracted using a Soxhlet apparatus. The resulting extracts were concentrated with a rotary evaporator and subsequently dried in a Plus11 Gallenkamp oven at 45°C. The dried extracts were then stored in a refrigerator at 4°C until administration to the rats (35).

Animal Husbandry and Experimental Design

Thirty-five adult male Wistar rats weighing an average of 200g were purchased from the animal holding of the University of Medical Sciences, Ondo, Ondo State, Nigeria (UNIMED). They were fed with pellets that were obtained from the local market in Ondo town, Ondo State. The rats were properly housed in a well-ventilated and clean space at the Animal House at UNIMED and acclimatised for 2 weeks before the commencement of exposure and treatment. Cadmium chloride (CdCl_2) solution, as previously described (33, 34), was administered weekly at a standard dose of 30mg/kg/BW across the test groups. In comparison, 200mg/kg/BW of each plant extract was administered, singly and combined, to the exposed and treated groups, respectively, for 21 days orally via gavage. The rats were grouped randomly into five groups, with each consisting of seven rats as described in Table 1.

Table 1: Animal grouping and treatment

Animal Group	Treatment
Group 1	The unexposed control group was administered distilled water only
Group 2	CdCl_2 - exposed untreated group
Group 3	CdCl_2 - exposed, <i>A. melegueta</i> treated group at 200mg/kg/BW
Group 4	CdCl_2 - exposed, <i>R. vomitoria</i> treated group at 200mg/kg/BW
Group 5	CdCl_2 -exposed, combined treatment with <i>R. vomitoria</i> and <i>A. melegueta</i> at 200mg/kg /BW, respectively.

Histopathological Studies

Histological examination of the testis was done using the Haematoxylin and Eosin (H & E) staining technique (18). The testes of rats across all groups were adequately fixed and processed (36). Briefly, the testes of the rats were excised and fixed using Bouin's fluid, dehydrated with grades of alcohol, and cleared in two changes of xylene, then embedded in blocks of paraffin and sectioned at

4 μm (37). Stained sections were studied microscopically for histopathological changes using the Olympus CX33 trinocular microscope, and photomicrographs were obtained using a Kodak PIXPRO A2527 with a megapixel of 20.68MP digital camera with a 1/2.3" BSI CMOS Sensor.

Histological Morphometric Analysis

Spermatogenesis and spermatogenic activity were evaluated histopathologically and graded using Johnsen's scoring system (38). By using a $\times 20$ magnification, thirty-five seminiferous tubules were randomly selected and evaluated per animal. Each

tubule was assigned a score ranging from 1 to 10 based on the presence or absence of specific germ cell types in sequential order of maturation, following Johnsen's scoring system, as detailed in Table 2.

Table 2: Johnsen's Scoring System for Evaluation of Spermatogenesis in Seminiferous Tubules

Scoring	Indication(s)
Score 10	Full spermatogenesis (germ cells at different developmental stages, spermatid)
Score 9	Many late spermatids, but dislodged tubular epithelium with significant sloughing or closing of the central cavity
Score 8	Only a few late spermatids
Score 7	No late spermatids, but many early spermatids
Score 6	Only a few early spermatids
Score 5	No spermatids, or spermatozoa, but many spermatocytes present
Score 4	No spermatids, few spermatocytes, and arrest of spermatogenesis at the primary spermatocyte stage
Score 3	Only spermatogonia cells are present.
Score 2	No germ cells, only Sertoli cells present
Score 1	Absence of seminiferous epithelial cells with the presence of tubular sclerosis

A higher Johnsen score (10) indicates full maturation with maximum activity, while a score of 1 means no maturation of sperm cells

Expression of Myeloperoxidase (MPO)

Total RNA was extracted from tissue samples using the Quick-RNA MiniPrepTMM kit (Zymo Research). DNA contamination was eliminated through treatment with DNAase I (NEB.Cat: M03035). Quantification of RNA at 260nm was carried out, and the purity was verified at both 260nm and 280nm using the A&E spectrophotometer (AcE Lab. UK). One microgram (1 μ g) of DNA-free RNA was converted into cDNA through a reverse transcriptase reaction using the cDNA synthesis kit based on protoScript II first-strand technology (New England BioLabs). This conversion process involved a 3-step reaction: 65°C for 5 minutes, 42°C for 1 hour, and 80°C for 5 minutes (39). The gene of interest was amplified using Polymerase Chain Reaction (PCR) with OneTaqR2X Master Mix (NEB) and primers

(Ingaba Biotech, Hatfield, South Africa). The PCR reaction was conducted in a 25 μ l volume mixture containing primer (forward and reverse) and Ready Mix Taq PCR master mix. The PCR amplification was performed under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of amplification (denaturation at 95°C for 30 seconds, annealing for 30 seconds and extension at 72 °C for 60 seconds) and ending with a final extension at 72°C for 10 minutes. The amplicons were resolved on a 1.0% agarose gel. The GAPDH gene was used to normalise the relative level of expression of each gene, and quantification of band intensity was done using "Image J" software following a previous study by Moronkeji et al. (34). Details of the primer used are shown in Table 3.

Table 3: Primer details

Primer	Primer sequence	Primer Sequences
Myeloperoxidase (MPO)	Forward	5'- GGCATCACTACCGTGTCTAA- 3'
	Reverse	5'- CCAGGAAGCCAGATTCAGTT- 3'
GAPDH	Forward	5'-AGACAGCCGCATCTTCTTGT-3'
	Reverse	5'-CTTGCCGTGGGTAGAGTCAT-3'

Statistical Analysis

The data were statistically analysed using the Statistical Package for the Social Sciences (SPSS), version 23. The one-way analysis of variance (ANOVA) was applied to compare categorical variables, and P-values ≤ 0.05 were considered statistically significant with a confidence level of 95%.

Results

mRNA Expression

Figure 1 presents the comparative analysis of MPO gene expression across the experimental groups. MPO expression was significantly upregulated in the CdCl₂-exposed untreated rats relative to the unexposed control group ($p < 0.05$). Relative to the

exposed untreated group, MPO expression remained elevated in rats treated singly with *A. melegueta* ($p < 0.05$) and *R. vomitoria* ($p > 0.05$). Further intergroup comparison revealed a significantly lower MPO expression in the *R. vomitoria*-treated group compared with the *A.*

melegueta-treated group ($p < 0.05$). Notably, co-administration of both extracts produced the greatest, although non-significant, downregulation of MPO expression relative to all other treatment groups ($p > 0.05$).

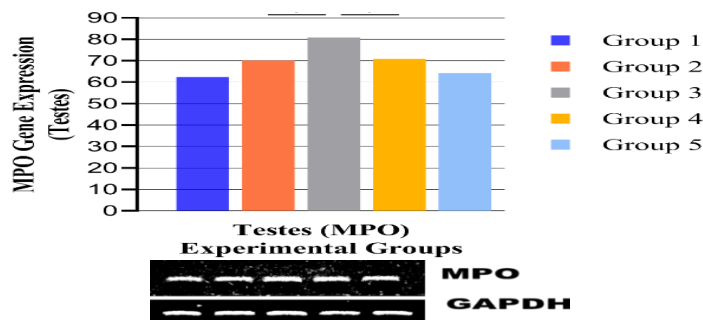


Figure 1: The effect of treatment paradigms on testicular MPO expression levels in experimental animals

The mRNA expression of MPO was significantly increased across Groups 2 - 4 when compared to the unexposed control ($p < 0.05$). A reduction was observed in the co-administrative treatment group, while other groups were statistically insignificant relative to the CdCl₂-exposed untreated rats ($p > 0.05$).

Histopathological Findings

The histopathological findings in this study demonstrated the attenuating effect of *R. vomitoria* and *A. melegueta* on cadmium-induced testicular toxicity in adult Wistar rats by ameliorating the deleterious impact of cadmium on the seminiferous tubules of the rats administered with the methanolic extracts of the studied plants. Histoarchitectural studies of the unexposed control rats exhibited normal histological features, characterised by full spermatogenesis typified by the presence of various cell types, including the germ cells, Sertoli cells, spermatogonia, spermatocytes, spermatids (early and late), connective tissue, and interstitial Leydig cells. The tissue morphology appeared normal and was devoid of any pathological lesions, as shown in Figure 2a. Figure 2b shows the CdCl₂-exposed untreated rats showing a moderately poor morphology typified by tubular distortion in the seminiferous tubules with dislodgement of germ cells from the basal epithelium as well as spermatogenic arrest. The interstitial connective

tissues appeared loose, while a few seminiferous tubules appeared normal. Figure 2c shows that CdCl₂-exposed rats administered with *A. melegueta* showed normal repair with the restoration of normal histological features of different germinal layers of maturing spermatogonia cells, while spermatogenic arrest is evident in some tubules, with the venule and connective tissue being evident. Figure 2d shows the CdCl₂-exposed administered with *R. vomitoria*, revealing distorted germ cells with other seminiferous tubules containing the terminal stage of the spermatogenic cycle and all cell types appearing normal, and some showing different germinal layers of maturing spermatogonia cells. The CdCl₂-exposed rats treated with combined extract of *A. melegueta* and *R. vomitoria* showed a distorted germ cell layer, the morphology of some of the seminiferous tubules appeared normal, interstitial Leydig cells and blood vessels were evident, while interstitial connective tissue appeared loose (Figure 2e).

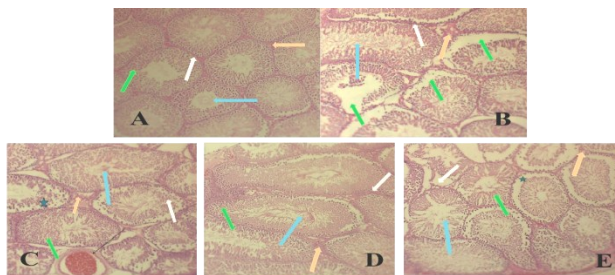


Figure 2: H and E-stained testicular sections across all groups

a. Control rats; seminiferous tubule containing the terminal stage of the spermatogenic cycle (blue arrow), all cell types are present, including Sertoli cells, spermatogonia, and spermatocytes. The green arrow shows the different germinal layers of maturing spermatogonia cells. The interstitial space consists of Leydig cells (orange arrow) while the connective tissues (white arrow) appear normal.

b. CdCl₂-exposed untreated rats, showing distorted interstitial connective tissue (orange arrow) with distorted germ cell layer (green arrow). Some of the seminiferous tubules appear normal, consisting of the terminal stage of the spermatogenic cycle with all cell types present, including Sertoli cells, spermatogonia, and spermatocytes (starred).

c. CdCl₂ + *A. melegueta* treated rats; the terminal stage of the spermatogenic cycle (blue arrow) in all cell types. The green arrow shows the different germinal layers of maturing spermatogonia cells, while spermatogenic arrest is evident in some tubules (starred). The venule (slender arrow) and connective tissue (white arrow) are evident.

d. CdCl₂ + *R. vomitoria* treated rats; distorted germ cells (starred) with other seminiferous tubules containing the terminal stage of the spermatogenic cycle (blue arrow), all cell types with some showing the different germinal layers of maturing spermatogonia cells (green arrow). Interstitial Leydig cells (orange arrow), connective tissue septa (white arrow), and blood vessels (slender arrow) appeared normal.

e. CdCl₂-exposed + combined treatment with *R. vomitoria* and *A. melegueta* at 200mg/kg /BW: distorted germ cell layer (green arrow). Some of the seminiferous tubules appear normal, consisting of the terminal stage of the spermatogenic cycle with all cell types present, including Sertoli cells, spermatogonia, and spermatocytes (green arrow). The interstitial connective tissue is loose (white arrow) while interstitial Leydig cells (orange arrow) and blood vessels (slender) are evident. (Magnification x100).

Johnsen's Score Analysis

The box and whisker plot in Figure 3 shows Johnsen's score of the seminiferous tubules of each group of experimental rats. Johnsen's score in the control group was higher compared to the other groups. The result showed that there was a deleterious impact of CdCl₂ on testicular structure and spermatogenesis in adult male Wistar rats in the exposed untreated rats when compared to the control group. Values of the seminiferous tubule count in the control section were significantly higher; the majority of seminiferous tubule values are above the median score. Whereas the exposed untreated group had most of the seminiferous tubule values below the median range, which was significantly lower. However, the group singly treated had the majority of their seminiferous tubule value below the median when compared to the exposed, untreated rats. The combined extracts treated group had most of the seminiferous tubule values below the median range.

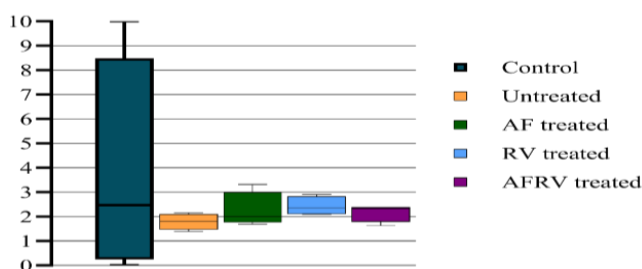


Figure 3: Whisker and Box plot of Johnsen's score for the Histoarchitecture of the testes

Discussion

Cadmium is a known environmental toxicant and endocrine disruptor in humans, affecting organs such as the kidneys, liver, and testes. Reports have identified the testis as particularly sensitive to cadmium toxicity, leading to irreversible morphological and functional alterations in the seminiferous epithelium with oxidative stress, inflammation, and apoptosis implicated in these deleterious effects (40, 41). Heavy metal or excessive environmental pollutants can harm the male reproductive system, highlighting the need for

research on the negative impacts of cadmium. The histological results of this study revealed that the cadmium-exposed untreated rats showed evident tubular distortion in the seminiferous tubules, alterations in the organisation of spermatogenic layers, Leydig cell degeneration, and congested interstitial layers compared to the unexposed control rats, characterised by unaltered histoarchitecture, and were devoid of any pathological lesions. The findings in this study are in tandem with the report by Sakr et al. (42) who observed that oral administration of cadmium at a

dose of 30mg/kg body weight induces testicular morphological alteration. These changes have been linked to cadmium-induced oxidative stress, resulting in reduced antioxidant defences and increased lipid peroxidation levels (43, 44). Furthermore, the group exposed to cadmium and treated with *A. melegueta* showed partial restoration of testicular histoarchitecture compared to the CdCl₂-exposed untreated group. This was marked by the presence of various germinal layers of maturing spermatogonia, although spermatogenic arrest remained evident in some tubules. Previous studies by Ngwoke *et al.* (27) and Chikere *et al.* (45) showed that significant medicinal plant properties can be utilised to treat various diseases. The protective or antimutagenic, anti-inflammatory impacts have been ascribed to various categories of plant phytochemical components (20), having some plant seeds showing strong anti-inflammatory potentials that may be useful in treating acute and chronic inflammation and some metabolic diseases (46). Similar findings have been reported in studies involving *A. melegueta* treatment against paraquat-induced toxicity, where histological investigations revealed improved testicular morphology following administration of the extract, consistent with the observations of Chikere *et al.* (45). In contrast, the *R. vomitoria*-treated group exhibited a more preserved histoarchitectural structure compared to the CdCl₂-exposed untreated and the other treatment groups. Our findings in this study are consistent with previous studies showing that *R. vomitoria* has the potential to improve male fertility and address reproductive issues (22). This plant plays a key role in spermatogenesis, a process that includes spermatogonia growth, meiosis, and spermatid maturation. Disruptions in this process, often due to damage to Sertoli cells or seminiferous tubules, can lead to infertility. Similarly, Lembe *et al.* (15) reported that the administration of a high dose (200 mg/kg/BW) of aqueous *R. vomitoria* extract significantly increased sperm count, further supporting its regulatory role in the spermatogenic process. However, this study revealed that co-administration of *A. melegueta* and *R. vomitoria* resulted in loosened interstitial connective tissue, a distorted germ cell layer, with only a few seminiferous tubules appearing normal. Further investigation into the underlying mechanisms may provide greater insight into the potential synergistic factors contributing to these effects. The histological grading system, like the Johnsen scoring system, serves as a dependable, readily accessible, and straightforward tool for predicting reproductive potential in men (47). The histological

evaluation of seminiferous tubules in this study indicated that most of the seminiferous tubule values observed in the cadmium-exposed group were below the median range. However, the score was higher in the singly treated group relative to the exposed untreated group. The most significantly observed value was evident in the control group, with the values higher than the median range, which indicated a better status of spermatogenesis. The singly treated group was significantly reduced when compared to the control group. In this study, Johnsen's score showed that *R. vomitoria* exhibited a superior and more effective treatment effect on cadmium when compared to the other treatment group. Consistent with the findings in this study, Similar reports about Johnsen's score as a method that determines the morphology of the cells in the seminiferous tubules report that Johnsen's score can be derived from testicular histopathological techniques, which can serve as a reliable tool to predict enhancements in the reproductive study of infertility (48). In a study by Thanh *et al.* (49), Johnsen's scoring system revealed a significant assessment of murine testicular histopathology in seminiferous tubules. Our earlier study confirmed that both *A. melegueta* and *R. vomitoria* contain a range of bioactive phytochemicals, including alkaloids, terpenoids, flavonoids, and saponins, as revealed through quantitative analysis and HPLC profiling (34). These findings are consistent with the phytochemical investigation of aqueous stem bark extract, which also identified polyphenols and alkaloids known for their anti-inflammatory and antioxidant properties (50). The mRNA expression in this study showed a significant elevation in myeloperoxidase (MPO) activity in the Cadmium exposed-untreated group (group 2) when compared to the control group, which is in agreement with the report by Abarikwu *et al.* (51), who indicated that, MPO poses impairment in their expression and represents a potential risk for tissue damage due to inflammation further substantiating our findings in this study as elevated MPO activity was observed to be associated with testicular distortion in the CdCl₂-exposed untreated rats. The CdCl₂-exposed rats treated with *A. melegueta* alone exhibited a significant upregulation of MPO expression compared to the untreated CdCl₂-exposed group. Interestingly, co-administration of *A. melegueta* and *R. vomitoria* resulted in a non-significant downregulation of MPO expression when compared to the groups treated with either extract alone. The significant repression of MPO expression suggests a potential synergistic interaction between the anti-

inflammatory properties of the studied plants. The findings of this study could be harnessed and applied for therapeutic applications and as a futuristic direction in the research and the management of heavy metal-induced toxicity of the reproductive system, as reported in some studies on selected organs (52, 53). This highlights the promise of *A. melegueta* and *R. vomitoria* as natural agents for mitigating the inflammatory responses associated with cadmium exposure.

Conclusion

Medicinal plants have demonstrated significant potential as natural remedies for various health conditions. This research highlights their crucial role in mitigating CdCl₂-induced testicular damage. The treatment with either extract of *A. melegueta* and *R. vomitoria*, as well as their coadministration, improved seminiferous tubular histoarchitecture relative to the CdCl₂-exposed untreated rats. Analysis of myeloperoxidase mRNA expression further revealed that co-administrative treatment more effectively downregulated testicular MPO expression levels than single extract treatment; these findings provide hypothesis-generating evidence that methanolic leaf extracts of *A. melegueta* and *R. vomitoria* are associated with attenuation of key indicators of CdCl₂-induced testicular toxicity in Wistar rats and indicate that the coadministration of the extracts of the two plants may offer protection against cadmium-induced testicular alterations

Recommendation

Future research should prioritise chronic studies to assess the long-term impact of cadmium exposure on testicular function and the efficacy of the plant extracts in reversing the induced damage over time. In addition to histopathological analysis, incorporating advanced techniques such as biochemical assays, hormonal assessments, seminal fluid analysis, and imaging techniques would provide a more comprehensive evaluation of the reproductive studies.

Declarations

Ethics Approval and Consent to Participate

Ethical approval was sought and obtained from the University of Medical Sciences Ethical Committee with a reference number-UNIMED-AREC/Apv/2024/010.

Consent for publication

All authors gave consent for publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

All essential data supporting the findings of this case are available within the article. Additional data are available upon request from the corresponding author.

Conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Authors' contributions

TA, AM: Conceptualisation, Data collection, methods and interpretation, statistical analysis of data, and writing of the initial and final draft of the article.

TA, AM, KA, AOM, KI: conducted research, gathered, arranged, analysed, and interpreted data, and critically examined and approved the final document.

AM, KA: Manuscript writing and similarity index.

Acknowledgements

Nil.

References

1. Bharti R, Sharma R. Effect of heavy metals: An overview. *Materials Today: Proceedings*. 2022 Jan 1;51:880-5. <https://doi.org/10.1016/j.matpr.2021.06.278>
2. Moronkeji A, Olayanju A, Adeniyi TD, Atere AD, Moronkeji AI, Igunbor MC, Oyeleke A, Akinbo FO. Oxidative stress response to gasoline generator exhaust emission in adult male wistar rats. *Environmental Analysis, Health and Toxicology*. 2024 Dec 26;39:e2024030. <https://doi.org/10.5620/eaht.2024030>
3. Mitra S, Chakraborty AJ, Tareq AM, Emran TB, Nainu F, Khusro A, Idris AM, Khandaker MU, Osman H, Alhumaydhi FA, Simal-Gandara J. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University-Science*. 2022 Apr 1;34(3):101865. <https://doi.org/10.1016/j.jksus.2022.101865>
4. Skolarczyk J, Budzynski M, Pekar J, Malecka-Massalska T, Skorzynska-Dziduszko K. The impact of cadmium on male infertility. *Journal of Elementology*. 2018;23(1). <https://doi.org/10.5601/jelem.2017.22.2.1320>

5. Unsal V, Dalkıran T, Çiçek M, Kölükçü E. The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. *Advanced Pharmaceutical Bulletin*. 2020 Feb 18;10(2):184. <https://doi.org/10.34172/apb.2020.023>
6. Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*. 2020 Jun;17(11):3782. <https://doi.org/10.3390/ijerph17113782>
7. Chien SH, Carmona G, Prochnow LI, Austin ER. Cadmium Availability from Granulated and Bulk-Blended Phosphate-Potassium Fertilizers. *Journal of Environmental Quality*. 2003 Sep;32(5):1911-4. <https://doi.org/10.2134/jeq2003.1911>
8. Shaari NE, Tajudin MT, Khandaker MM, Majrashi A, Alenazi MM, Abdullahi UA, Mohd KS. Sintomas de toxicidade de cádmio e mecanismo de absorção em plantas: uma revisão. *Brazilian Journal of Biology*. 2024;84:e252143. <https://doi.org/10.1590/1519-6984.252143>
9. Kim TH, Kim JH, Le Kim MD, Suh WD, Kim JE, Yeon HJ, Park YS, Kim SH, Oh YH, Jo GH. Exposure assessment and safe intake guidelines for heavy metals in consumed fishery products in the Republic of Korea. *Environmental Science and Pollution Research*. 2020 Sep;27(26):33042-51. <https://doi.org/10.1007/s11356-020-09624-0>
10. Sarkar A, Ravindran G, Krishnamurthy V. A brief review on the effect of cadmium toxicity: from cellular to organ level. *Int J Biotechnol Res*. 2013;3(1):17-36.
11. Mahmoudi R, Azizi A, Abedini S, Jahromi VH, Abidi H, Barmak MJ. Green tea improves rat sperm quality and reduced cadmium chloride damage effect in spermatogenesis cycle. *Journal of Medicine and Life*. 2018 Oct;11(4):371. <https://doi.org/10.25122/jml-2018-0005>
12. Zhu Q, Li X, Ge RS. Toxicological effects of cadmium on mammalian testis. *Frontiers in Genetics*. 2020 May 26;11:527. <https://doi.org/10.3389/fgene.2020.00527>
13. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*. 2017;2017(1):8416763. <https://doi.org/10.1155/2017/8416763>
14. Gulumian M, Yahaya ES, Steenkamp V. African herbal remedies with antioxidant activity: a potential resource base for wound treatment. *Evidence-Based Complementary and Alternative Medicine*. 2018;2018(1):4089541. <https://doi.org/10.1155/2018/4089541>
15. Lembe DM, Koloko BL, Bend EF, Domkam J, Oundoum PC, Njila MN, Moundipa P, Dimo T, Gonzales GF. Fertility enhancing effects of aqueous extract of *Rauvolfia vomitoria* on reproductive functions of male rats. *J Exp Integr Med*. 2014 Jan 1;4(1):43-9. <https://doi.org/10.5455/jeim.200913.or.090>
16. Ikokide EJ, Oyagbemi AA, Oyeyemi MO. Impacts of cadmium on male fertility: Lessons learnt so far. *Andrologia*. 2022 Oct;54(9):e14516. <https://doi.org/10.1111/and.14516>
17. Dutta S, Sengupta P, Slama P, Roychoudhury S. Oxidative stress, testicular inflammatory pathways, and male reproduction. *International Journal of Molecular Sciences*. 2021 Sep 17;22(18):10043. <https://doi.org/10.3390/ijms221810043>
18. Nna VU, Ujah GA, Mohamed M, Etim KB, Igba BO, Augustine ER, Osim EE. Cadmium chloride-induced testicular toxicity in male wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal. *Biomedicine & Pharmacotherapy*. 2017 Oct 1;94:109-23. <https://doi.org/10.1016/j.biopha.2017.07.087>
19. Kehinde AO, Ekundina VO, Iyiola S, Avwioro G, Enye LA. Synergistic nutraceutical effects of aqueous extracts from *Vernonia amygdalina* and *Moringa oleifera*: A promising therapeutic strategy for gastric ulcer management. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine*. 2024 Oct 1;3(4):296-302. https://doi.org/10.4103/jpdtm.jpdtm_102_24
20. Ajayi OA. Phytochemical and GC-MS analysis of bioactive components in ethanolic extract of *Rauvolfia vomitoria* leaves. *Journal of Chemical Society of Nigeria*. 2021 Sep 6;46(4). <https://doi.org/10.46602/jcsn.v46i4.642>
21. Akpojotor P, Ebomoyi MI. Effect of hydromethanolic extract of *Rauvolfia vomitoria* leaf on blood glucose, plasma insulin and histomorphology of the pancreas of streptozotocin-induced diabetic male wistar rats. *Journal of African Association of Physiological Sciences*. 2021 Aug 3;9(1):40-7.
22. Koloko BL, Bushra I, Wankeu-Nya M, Ngaha Njila MI, Kenmogne H, Nyonseu Nzeubang DC, Nzangueu CB, Dimo T, Dongmo AB, Massoma Lembe D. In vivo effects of *Rauvolfia vomitoria* (Apocynaceae) ethanolic extract on sexual

- performance and reproductive activity in male rats. *Andrologia*. 2020 Feb;52(1):e13414. <https://doi.org/10.1111/and.13414>
23. Ezejindu DN, Asomugha AL, Ukoha U. Hepatoprotective effects of *Rauwolfia vomitoria* extract on the liver of adult wistar rats. *IJPR*. 2013;3(4):71.
24. Owaba AD, Rafiu RO, Frank A, Eboh DD, Bunu SJ. Isolation of Novel 6-methylideneoxecane-3, 4, 5, 7, 8, 9-hexol from the Leaves of *Rauwolfia vomitoria*, Apocynaceae. *Biology, Medicine, & Natural Product Chemistry*. 2024 May 22;13(1):135-9. <https://doi.org/10.14421/biomedich.2024.131.135-139>
25. Oyinloye BE, Ajiboye BO, Ojo OA, Musa HM, Onikanni SA, Ojo AA. Ameliorative potential of *Aframomum melegueta* extract in cadmium-induced hepatic damage and oxidative stress in male Wistar rats. 2016;6(7):1-6. <https://doi.org/10.7324/JAPS.2016.60714>
26. Nwozo SO, Yakubu OF, Oyinloye BE. Protective Effect of Aqueous Extracts of *Aframomum Melegueta* on Γ -Radiation-Induced Liver Damage in Male Wistar Rats. *Mil. Med. Sci. Lett.*. 2013 Sep 6;82(3):126-32. <https://doi.org/10.31482/mmsl.2013.018>
27. Ngwoke KG, Chevallier O, Wirkom VK, Stevenson P, Elliott CT, Situ C. In vitro bactericidal activity of diterpenoids isolated from *Aframomum melegueta* K. Schum against strains of *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. *Journal of Ethnopharmacology*. 2014 Feb 12;151(3):1147-54. <https://doi.org/10.1016/j.jep.2013.12.035>
28. Ogwu MC, Dunkwu-Okafor A, Omakor IA, Izah SC. Medicinal spice, *Aframomum melegueta*: An overview of the phytochemical constituents, nutritional characteristics, and ethnomedicinal values for sustainability. *Herbal Medicine Phytochemistry: Applications and Trends*. 2024 Jul 10:221-43. https://doi.org/10.1007/978-3-031-43199-9_72
29. Loria V, Dato I, Graziani F, Biasucci L. Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. *Mediators of Inflammation*. 2008;2008(1):135625. <https://doi.org/10.1155/2008/135625>
30. Ali M, Jasmin S, Fariduddin M, Alam SM, Arslan MI, Biswas SK. Neutrophil elastase and myeloperoxidase mRNA expression in overweight and obese subjects. *Molecular Biology Reports*. 2018 Oct;45(5):1245-52. <https://doi.org/10.1007/s11033-018-4279-4>
31. Pullar JM, Carr AC, Bozonet SM, Rosengrave P, Kettle AJ, Vissers MC. Elevated seminal plasma myeloperoxidase is associated with a decreased sperm concentration in young men. *Andrology*. 2017 May;5(3):431-8. <https://doi.org/10.1111/andr.12327>
32. Onoja SO, Omeh YN, Ezeja MI, Chukwu MN. Evaluation of the in vitro and in vivo antioxidant potentials of *Aframomum melegueta* methanolic seed extract. *Journal of Tropical Medicine*. 2014;2014(1):159343. <https://doi.org/10.1155/2014/159343>
33. Adeniyi T, Akinpelu M, Akinlami O, Kolawole MJ, Oloye J, Akogun I. Attenuation of cadmium toxicity by methanol extracts of *Rauwolfia vomitoria* and *Aframomum melegueta* leaves. *Folia Medica Indonesiana*. 2024;60(2):94-102. <https://doi.org/10.20473/fmi.v60i2.55646>
34. Moronkeji A, Adeniyi T, Olubunmi E, Kayode I, Ajala OJ, Moronkeji AI, Idowu D. Modulatory effects of *Aframomum melegueta* and *Rauwolfia vomitoria* in cadmium-induced renal toxicity in adult rats. *Journal of Cellular Biotechnology*. 2025 Feb;11(1):3-13. <https://doi.org/10.1177/23523689251329719>
35. Morakinyo AE, Oyedapo OO, Akinpelu BA, Fajobi AO, Fasakin WO, Komolafe IJ, Akinlalu AO. Evaluation of antioxidant, anti-inflammatory and phytochemical constituents of *Aframomum melegueta* aqueous leaf extract. *International Journal of Medicinal Plants*. 2018;112:888-903.
36. Akinpelu M, Gerald EI, Ayodele BR, Akpu AO, Chuks IM. Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats. *Journal of Medicinal Plants Research*. 2018 Nov 25;12(28):508-21. <https://doi.org/10.5897/JMPR2018.6661>
37. Mohamed M, Sulaiman SA, Jaafar H, Sirajudeen KN. Antioxidant protective effect of honey in cigarette smoke-induced testicular damage in rats. *International Journal of Molecular Sciences*. 2011 Aug 29;12(9):5508-21. <https://doi.org/10.3390/ijms12095508>
38. Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormone Research in Paediatrics*. 1970 Nov 21;1(1):2-5. <https://doi.org/10.1159/000178170>
39. Elekofehinti OO, Onunkun AT, Olaleye TM. *Cymbopogon citratus* (DC.) Stapf mitigates ER-stress induced by streptozotocin in rats via down-regulation of GRP78 and up-regulation of Nrf2 signaling. *Journal of Ethnopharmacology*. 2020 Nov 15;262:113130. <https://doi.org/10.1016/j.jep.2020.113130>

40. Siu ER, Mruk DD, Porto CS, Cheng CY. Cadmium-induced testicular injury. *Toxicology and Applied Pharmacology*. 2009 Aug 1;238(3):240-9. <https://doi.org/10.1016/j.taap.2009.01.028>
41. Ferlazzo N, Micali A, Marini HR, Freni J, Santoro G, Puzzolo D, Squadrito F, Pallio G, Navarra M, Cirmi S, Minutoli L. A flavonoid-rich extract from bergamot juice, alone or in association with curcumin and resveratrol, shows protective effects in a murine model of cadmium-induced testicular injury. *Pharmaceuticals*. 2021 Apr 21;14(5):386. <https://doi.org/10.3390/ph14050386>
42. Sakr SA, Nooh HZ. Effect of Ocimum basilicum extract on cadmium-induced testicular histomorphometric and immunohistochemical alterations in albino rats. *Anatomy & Cell Biology*. 2013 Jun 30;46(2):122. <https://doi.org/10.5115/acb.2013.46.2.122>
43. Han C, Zhu Y, Yang Z, Fu S, Zhang W, Liu C. Protective effect of Polygonatum sibiricum against cadmium-induced testicular injury in mice through inhibiting oxidative stress and mitochondria-mediated apoptosis. *Journal of Ethnopharmacology*. 2020 Oct 28;261:113060. <https://doi.org/10.1016/j.jep.2020.113060>
44. Ibrahim NM, Kandil AM, Ali RS, Hesham R, El-Ashram S, Alquraishi MI, Abdel-Gaber R, Dkhil MA, Elhemily AA. Zeólito anula danos testiculares induzidos por cádmio em ratos: implicação da via NF-κB/TNF-α/IL-1β. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 2024;76:e13194. <https://doi.org/10.1590/1678-4162-13194>
45. Chikere OU, Uchechi EE, Ikenna IM, Ndubuisi OA. Ameliorative effect of Aframomum Melegueta (alligator pepper) against paraquat-induced testicular damage. *World Journal of Pharmaceutical Research*. 2020 Mar 18;9:2105-24.
46. Ilic N, Schmidt BM, Poulev A, Raskin I. Toxicological evaluation of grains of paradise (Aframomum melegueta)[Roscoe] K. Schum. *Journal of Ethnopharmacology*. 2010 Feb 3;127(2):352-6. <https://doi.org/10.1016/j.jep.2009.10.031>
47. Dohle GR, Elzanaty S, Van Casteren NJ. Testicular biopsy: clinical practice and interpretation. *Asian Journal of Andrology*. 2011 Dec 12;14(1):88. <https://doi.org/10.1038/aja.2011.57>
48. Teixeira TA, Pariz JR, Dutra RT, Saldiva PH, Costa E, Hallak J. Cut-off values of the Johnsen score and Copenhagen index as histopathological prognostic factors for postoperative semen quality in selected infertile patients undergoing microsurgical correction of bilateral subclinical varicocele. *Translational Andrology and Urology*. 2019 Aug;8(4):346. <https://doi.org/10.21037/tau.2019.06.23>
49. Thanh TN, Van PD, Cong TD, Le Minh T, Vu QH. Assessment of testis histopathological changes and spermatogenesis in male mice exposed to chronic scrotal heat stress. *Journal of Animal Behaviour and Biometeorology*. 2020 Oct 19;8(3):174-80. <https://doi.org/10.31893/jabb.20023>
50. Bonheur YD, Désiré DD, Ernestine NZ, Stéphane Z, Christophe M, Oumarou BF, Francine TN, Anatole PC, Théophile D. Antiinflammatory and antioxidant effects of the stem bark aqueous extract of Rauwolfia vomitoria (Apocynaceae) in female Wistar rats. *European Journal of Pharmaceutical and Medical Research*. 2015;2:64-73.
51. Abarikwu SO, Oruitemeka S, Uwadileke IA, Omeodu SI, Okoye NF, Mgbudom-Okah CJ, Ohanador R. Oral administration of cadmium depletes intratesticular and epididymal iron levels and inhibits lipid peroxidation in the testis and epididymis of adult rats. *Journal of Trace Elements in Medicine and Biology*. 2018 Jul 1;48:213-23. <https://doi.org/10.1016/j.jtemb.2018.04.011>
52. Ogunbiyi OJ, Obi FO. Evaluation of gonadotoxic effects of cadmium and iron administered via tainted diet singly and combined in female rats. *Journal of Toxicology and Risk Assessment*. 2022 Dec 31;8(1):47. <https://doi.org/10.23937/2572-4061.1510047>
53. Ogunbiyi OJ, Obi FO. Evaluation of cadmium toxicity and its association with iron on the gonads of female rats. *Biokemistri*. 2021 Sep 30;33(3):169-80.