SSR Journal of Medical Sciences (SSRJMS)

Homepage: https://ssrpublisher.com/ssrjms/

Volume 3, Issue 9, 2026 ISSN: 3049-0375

Comparative Experimental Evaluation of *Rauwolfia vomitoria* and *Anthocleista vogelii* Extracts for Mitigating Radiation-Induced Testicular Endocrine Dysfunction and Histological Injury in Wistar Rats

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Received: *11.08.2026* | ***Accepted:*** *01.09.2026* | ***Published:*** *04.09.2026*

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DOI: *10.5281/zenodo.22288867*

Abstract **Original Research Article**

The surge in cancer incidence has increased use of radiation therapy. However, scattered or direct ionizing radiation to testicular tissue can impair steroidogenesis, gonadotropin control, and spermatogenesis. This study examined extracts of *Rauwolfia vomitoria* and *Anthocleista vogelii* as therapies for radiation-induced testicular injury.

We used thirty-six male Wistar rats randomly assigned to six groups of six. Group 1 served as control and received normal saline; Group II received 7-Gy abdominopelvic irradiation; Groups III and IV received 100mg/kg ethanolic extract of *Rauwolfia vomitoria* and 200mg/kg aqueous extract of *Anthocleista vogelii for* 14 days before irradiation, continued for eight weeks after irradiation. While Group V and VI received pre-treatment of the extract only.

After eight weeks, rats were euthanized, and blood and tissue specimens were collected for hormonal and histological examination. We assessed serum testosterone, oestradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), and analyzed histology. Compared with controls, irradiation reduced testosterone by 81%, oestradiol by 39.2%, and FSH and LH by 40.8% and 46.2%, respectively. Both extracts improved all hormone levels, $p < .001$. Histology assessment showed tubular necrosis, reduced germ cells, Leydig cell hypoplasia, and absent spermatogenesis; these changes correlated with lower hormone levels.

Treatment with extracts before and after irradiation increased hormones compared to irradiation alone and preserved seminiferous architecture and luminal spermatozoa. The *Rauwolfia vomitoria* groups did not differ from controls except for a decrease in testosterone level testosterone decrease. Pre-treatment with *Rauwolfia vomitoria* increased testosterone but did not restore FSH or LH. Continued treatment before and after irradiation produced the best endocrine and histological recovery, and was superior to pre-treatment alone. Further study using formulations, histology, mechanistic biomarkers, and fertility outcomes is needed.

Keywords: *Anthocleista vogelii*, ionizing radiation, reproductive hormones, *Rauwolfia vomitoria*, testicular damage.

Citation: Ajiboye, M. O., Olayinka, O. O., Usman, H. A., Animashaun, S. Y., & Oniye, F. B. (2026). Comparative experimental evaluation of *Rauwolfia vomitoria* and *Anthocleista vogelii* extracts for mitigating radiation-induced testicular endocrine dysfunction and histological injury in Wistar rats. *SSR Journal of Medical Sciences (SSRJMS)*, *3*(9), 1-18.

INTRODUCTION

Infertility is among the most critical reproductive health issues globally. Approximately 17.5% of the adult demographic is projected to encounter infertility at some stage in their lives (Cox et al., 2022; Mburu et al., 2026; World Health Organization [WHO], 2025). This condition emerges from a combination of therapeutic, lifestyle, nutritional, and socio-economic factors. Notably, male reproductive factors account for around 30–50% of infertility cases, underscoring the vital role of male fertility in global infertility (Agarwal et al., 2021; Eisenberg et al., 2023; Nixon et al., 2023). The rising global cancer burden has increased the demand for radiotherapy, which remains an essential treatment modality for approximately half of all patients with cancer worldwide (Abdel-Wahab et al., 2021, 2024; bray et al., 2024). Concurrently, radiotherapy presents a considerable threat to male fertility, despite its progress in oncological treatment (Elenkov & Giwercman, 2022; Su et al., 2025). Infertility caused by therapeutic radiation is promoted via a nonspecific cytotoxic mechanism. Radiation negatively impacts both malignant cells and the adjacent healthy tissues. The reproductive organs, especially the testes, demonstrate considerable sensitivity to radiation and can incur substantial harm when exposed. The testes is specifically susceptible to radiation due to the seminiferous epithelium, which contains rapidly dividing spermatogonial stem cells and germ cells. These cells undergo considerable apoptosis and cell cycle arrest due to oxidative stress caused by radiation treatment. Therapeutic scatter radiation, even in low doses, results in an increase in reactive oxygen species, lipid peroxidation, and considerable damage to seminiferous tubules. Pathological changes negatively influence spermatogenesis and the structural integrity of Sertoli and Leydig cells, while also influencing gonadotropin (FSH, LH) and testosterone concentrations (Elenkov & Giwercman, 2022; Fukunaga et al., 2022; Yumura et al., 2023). More than 30% of male cancer survivors experience a lasting reduction in fertility following radiotherapy to the lower body, spine, or pelvis. Recent studies have proposed several synthetic drugs to preserve testicular integrity and performance after radiation exposure. However, no ethnomedicinal remedy has been identified that can alleviate the initial radiation injury and promote recovery after exposure by preserving the structural and hormonal conditions essential for male fertility (Georgakopoulos et al., 2024; Mishra et al., 2025; Zhi et al., 2025).

The existing radioprotective approaches are limited and mostly concentrate on protecting fertility during or after irradiation, without considering the preservation of spermatogonia before significant damage occurs. A multitude of studies have identified synthetic radioprotectors that safeguard testicular architecture and alleviate oxidative stress and apoptosis, such as melatonin, amifostine, and dexmedetomidine (Dil et al., 2023; Farhadi et al., 2026; Mishra et al., 2025). Moreover, synthetic radioprotectors that affect antioxidant pathways, together with purified compounds such as coenzyme Q10, melatonin, and ferulic acid, have shown minimal protective advantages. Flora employed in ethnomedicine, featuring bioactive compounds such as alkaloids, flavonoids, saponins, and vitamins, has attracted attention due to their antioxidant, anti-inflammatory, and androgen-boosting properties. *Rauwolfia vomitoria* improved reproductive parameters, protected Leydig and Sertoli cells, and increased blood testosterone concentrations, whilst *Anthocleista vogelii* shown significant anti-inflammatory, antioxidant, and tissue-protective properties in animal research. However, these results are deficient in data to adequately tackle the considerable limitations of radioprotective therapy. Synthetic agents have not reliably overseen post-irradiation therapy and recovery. Systemic toxicity may limit the use of synthetic substances. Furthermore, the costs, negative consequences, and limited therapeutic windows inhibit the routine or extended application of pure compounds and synthetic radioprotective agents. The predominant studies have employed either a solitary synthetic substance or an individual natural extract. As a result, there are no comparative datasets available under the same experimental conditions. Nonetheless, the therapeutic microbiota may have a more extensive protective capability. Furthermore, experimental methodologies have generally encompassed either pre- or post-irradiation regimens, but not a synthesis of both that would

have provided radioprotection while aiding in the recovery of injured tissue. As a result, it is unclear which of the two native medicinal plants would demonstrate superior effectiveness under similar light exposure conditions, whether administering treatment prior to exposure differs from treatment after exposure, and whether the improvement of endocrine function is associated with the preservation or restoration of testicular histoarchitecture (Mishra et al., 2025; Roychoudhury et al., 2022; Yi et al., 2021). The present comparative examination of *Rauwolfia vomitoria*and *Anthocleista vogelii*, encompassing the assessment of pre-exposure treatment against a blend of pre- and post-exposure therapies, alongside the exploration of the correlation between endocrine outcomes and the maintenance or restoration of testicular architecture, is essential to rectify these shortcomings.

The present study aimed to fill the empirical gap by experimentally evaluating the biochemical and histological effects of ethanolic leaf extract from *Rauwolfia vomitoria* and aqueous leaf extract from *Anthocleista vogelii* on radiation-induced testicular damage in male Wistar rats. A dual strategy was utilized to assess whether these phytochemical-rich extracts given prior to exposure could reduce acute tissue injury (radioprotective effect) and whether their application post-irradiation could promote structural recovery and hormonal restoration (ameliorative effect). The study sought to evaluate the blood levels of gonadotropin, testosterone, and oestrogen, along with the qualitative testicular cytoarchitecture in adult male Wistar rats following a single abdominopelvic radiation exposure of 7.0 Gy. The study exhibited a deficiency in quantitative standardization for the chemical and phytochemical evaluation of the extract. The study offers significant clinical and pharmacological perspectives by delivering foundational comparative data that suggests ethnomedicinal plants, especially *Rauwolfia vomitoria*, could act as readily available and effective natural treatments for maintaining testicular health and attaining complete reproductive recovery post-radiotherapy.

2.0 METHODOLOGY

2.1 Experimental Design and Ethical Approval

Thirty-six male adult rats were procured from the Department of Anatomy, College of Medicine, University of Lagos, Idi-Araba, and Lagos, Nigeria and divided into six groups of six animals each. The experimental design consisted of a non-irradiated normal control, an irradiation-only control, two groups receiving extract before and after irradiation, and two groups given extract only before irradiation. This framework enabled the evaluation of irradiation impacts and comparison of each extract protocol against irradiation alone, as well as evaluating whether continued post-irradiation treatment led to better recovery than pre-treatment alone. Similar controlled strategies have been employed in evaluating protective measures against induced testicular damage (Abarikwu et al., 2022; Akhigbe et al., 2024; Ali et al., 2023; Askar et al., 2024). The study protocol was approved by the College Health Research Ethics Committee of the College of Medicine, University of Lagos (CMUL/HREC/08/21/906). All animal handling and experiment procedures were carried out according to the institutional regulations for the care and use of laboratory animals.

2.2 Experimental Animals

We used 36 adults' male Wistar rats weighing 180–210 g. These animals were procured from and housed in the Animal House of the Department of Anatomy, College of Medicine, and University of Lagos. The rats were housed in plastic cages with metal mesh lids in a well-ventilated chamber maintained at 26-29 °C after 12h light/dark cycle. The animals were allowed free access to standard rat food and fresh water and their cages were cleaned regularly. The therapy was started after a 14-day adaptation period of the animals. For a comprehensive evaluation of reproductive hormones, spermatogenesis, and testicular architecture, Wistar rats are commonly employed (Hariti et al., 2024; Srivastava et al., 2025). The animals were then acclimatized and randomly assigned into six experimental groups of six rats each.

Each rat represented a separate experimental unit. All groups were housed under similar housing and nutritional conditions during the course of the experiment.

2.3 Plant Collection and Extract Preparation

Leaves of *Rauwolfia vomitoria* were collected from the Botanical Garden, University of Lagos, Nigeria. The plant specimen was identified by a Botanist. The leaves were washed with running tap water and dried in an oven at 45–50 °C for three hours then ground in an electric blender. Leaves were powdered and soaked in ethanol for 24 hours, filtered and the solvent was allowed to evaporate at ambient temperature to obtain crude ethanolic leaf extract. Freshly harvested foliage of *Anthocleista vogelii* was desiccated in the air and ground with an electric blender (VR-BLO44B, Venus). One hundred grams of the powdered material was suspended in one litre of distilled water and allowed to stand with periodic stirring for 48 hours and filtered using Whatman filter paper. The filtrate was transferred to petri plates and dried in the oven at about 40 °C to obtain a crude aqueous extract.

Both formulations were used as crude extracts without chromatographic analysis or quantitative chemical standardization. The presence of antioxidant phytochemicals in *Rauwolfia vomitoria* leaves and oxido-inflammatory activity in *Anthocleista vogelii* were reported in earlier studies, which provide biological rationale for testing these extracts in radiation-induced tissue damage (Kpadonou-Kpoviessi et al., 2024; Oredeko et al., 2023).

2.4 Animal Grouping, interventions, and timeline

Group (n = 6)	Radiation	Oral intervention	Schedule and estimand
I: normal control	None	Physiological saline	Vehicle control across the experimental period
II: irradiation control	Single 7.0 Gy	No extract	Radiation injury relative to Group I
III: *Rauwolfia vomitoria* pre/post	Single 7.0 Gy	100 mg/kg ethanolic leaf extract	Pre-treatment; resumed 24 h after irradiation and continued for 8 weeks
IV: *Anthocleista vogelii* pre/post	Single 7.0 Gy	200 mg/kg aqueous leaf extract	Pre-treatment; resumed 24 h after irradiation and continued for 8 weeks
V: *Anthocleista vogelii* pre-only	Single 7.0 Gy	200 mg/kg aqueous leaf extract	Pre-treatment only
VI: *Rauwolfia vomitoria* pre-only	Single 7.0 Gy	100 mg/kg ethanolic leaf extract	Pre-treatment only

Table 2. Experimental groups and intended comparisons.

We administered the extracts once daily via oral gavage using an orogastric cannula. Groups III and VI received 100 mg/kg body weight of ethanolic *Rauwolfia vomitoria* leaf extract, while Groups IV and V received 200 mg/kg body weight of aqueous *Anthocleista vogelii* leaf

extract. The extracts were administered for 14 days prior to irradiation. Twenty-four hours post-irradiation, treatment was resumed in Groups III and IV, with daily administration continuing for eight weeks. Groups V and VI received only pre-treatment and no extract post-irradiation. Similar natural-product intervention studies have combined sustained oral treatment with endocrine and histological assessments of testicular injury (Abarikwu et al., 2022; Ahmed et al., 2021; Wang et al., 2021).

2.5 Procedure for Documentation of Irradiation and Dose

After the pre-treatment period, rats in Groups II–VI were anaesthetized with ketamine (50 mg/kg intramuscularly) and xylazine (5 mg/kg intraperitoneally). The three anaesthetized animals were placed in the supine position on the radiotherapy couch with the treatment field directed to the abdominopelvic region including the testes. A single fraction of 7.0 Gy was delivered with a static 6-MV photon beam. The treatment-console record showed 650 planned monitor units, a nominal dose rate of 600 MU/min, gantry and collimator angles of 0°, symmetric jaw positions of Y1 = 20.0 cm, Y2 = +20.0 cm, X1 = 20.0 cm, and X2 = +20.0 cm corresponding to a 40 40 cm field at the machine reference plane. Studies of radiation-induced testicular injury have shown a clear dose and dose-rate relationship of injury (Erdem et al., 2024; Farhadi et al., 2026; Spadella et al., 2026).

2.6 Blood collection, perfusion and tissue collection

After 8 weeks from irradiation and about 24 hours after the last administration of the extracts, the animals were anaesthetized with ketamine hydrochloride. The thoracic cavity was opened and blood was collected from the right ventricle into plain specimen bottles. We open the right atrium and insert a cannula into the left ventricle, flushing the system with normal saline to clear out all the circulating blood. We then perfused the tissue with 4% paraformaldehyde to fix the tissue. Testes were removed and fixed in Bouin's fluid for histological analysis. Hormonal analysis was done by separating serum from blood samples by centrifugation at 3000 rpm for 10 minutes.

2.6 Reproductive hormone assays in serum

Serum levels of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol were measured using enzyme-linked immunosorbent assay following the manufacturers' instructions. We used an LSBio rat FSH ELISA kit (LS-F6305) and a competitive rat LH ELISA kit (MBS729873). Samples and reagents were brought to the required assay temperatures, mixed gently and the controls and serum samples and standards were loaded into the designated wells. Testosterone and oestradiol assays were done in duplicate. The absorbance was read at 450 nm (reference wavelength 620–630 nm if necessary) and the concentrations of the hormones were calculated from the calibration curves. Serum testosterone, FSH, LH and oestradiol levels were used as complementary markers of hypothalamic-pituitary-gonadal and testicular endocrine function. Functional and structural parameters of testicular damage were assessed by reproductive hormone data and testicular morphology (Akhigbe et al., 2024; Ali et al., 2023; El-Naggar et al., 2022; Srivastava et al., 2025).

2.7 Histological preparation and Analysis

Testicular tissues fixed in Bouin's solution were dehydrated in a series of alcohol concentrations (50%, 70%, 90%, absolute I, and absolute II) for 1 hour each. The tissues were then cleared in two changes of xylene for 1 h each, infiltrated with molten paraffin wax at about 60 °C in two changes for 1 h each, embedded in paraffin and blocks trimmed. Sections of 7 µm were cut with a rotary microtome, floated on a water bath at ca 40 °C, mounted on glass slides and dried. Sections were deparaffinized with two changes of xylene, rehydrated through graded alcohols, stained with hematoxylin for 20 min, differentiated in 1% acid alcohol for 5 sec, and counterstained with eosin for 2 min. Sections were dehydrated, cleared and mounted in DPX . The prepared slides were observed under light microscope and photomicrographs were taken at

×100 and ×400 magnifications. H&E staining is a common method to demonstrate damage of seminiferous tubules and germinal epithelium in experimental studies of testicular injury (Ashour et al., 2025; Dil et al., 2023; Farhadi et al., 2026).

We studied the general structure of seminiferous tubules, the integrity and thickening of the basement membrane, the arrangement of germinal epithelium, the presence and development of spermatogenic cell populations, the morphology of Sertoli cells and Leydig cells, and the presence of spermatozoa in the tubular lumen. In addition, we evaluated vacuolation, cellular depletion, atrophy, necrosis and distortion of the seminiferous tubules. Qualitative comparison of histological findings was made among the six experimental groups. Histomorphological assessment has been shown to be a sensitive method to detect treatment-related testicular injury and tissue preservation in rodent models (Demirbağ et al., 2023; Hariti et al., 2024).

2.8 Statistical analysis

Each group consisted of six animals and each animal was considered as an independent experimental unit. Continuous reproductive hormone data are presented as mean ± standard error. Data distributions, model residuals and homogeneity of variance were examined prior to inferential analysis. One-way analysis of variance was performed to compare levels of testosterone, FSH, oestradiol and LH between the six groups, whereas Welch's ANOVA was employed for oestradiol and LH due to violation of the equal-variance assumption. Planned comparisons of each extract-treated group versus the irradiation-only group were performed using Dunnett-adjusted tests after one-way ANOVA, whereas Tukey-adjusted tests were used for other pairwise comparisons. Games–Howell post hoc test following Welch's ANOVA. The robustness of the results was assessed with the Kruskal – Wallis test followed by the pairwise comparison of Dunn with the Holm adjustment, where necessary. Holm correction was also performed across the four primary omnibus hormone tests.

Effect sizes were reported as eta-squared ($\eta 2$) and omega-squared ($\omega 2$), and classical variance-partition estimates were described for outcomes analysed with Welch's ANOVA. Pairwise effects were expressed as mean differences with simultaneous 95% confidence intervals and multiplicity-adjusted P values. All statistical tests were two-sided, and statistical significance was defined as an adjusted p-value < .05. Exact p values and number of animals in each analysis are reported where applicable. All analyses were performed in Python using the Google Colaboratory environment.

3.0 Results

Groups were defined as follows: Group 1 was the normal control; Group II was irradiated only; Group III was treated with *Rauwolfia vomitoria* before and after irradiation; *Anthocleista vogelii was* administered to Group IV before and after irradiation, to Group V as pre-treatment only and *Rauwolfia vomitoria* was administered as pre-treatment only to Group VI.

3.1 Concentration of Reproductive Hormones

Irradiation caused significant decreases in all four reproductive hormones. The mean concentrations in Group II were decreased by 81.0% for testosterone, 39.2% for oestradiol, 40.8% for FSH and 46.2% for LH compared to Group 1 (Table 1 and Figure 1). Overall group effects were significant for each outcome and remained significant after Holm correction across the four primary omnibus tests (see Table 2). In addition, rank-based sensitivity analyses identified overall group differences for all four hormones.

Table 1. *Reproductive hormone concentrations across the experimental groups*

Outcome	G1 Normal	G II IR	G III RV pre+post	G IV AV pre+post	G V AV pre	G VI RV pre
Testosterone (nmol/L)	3.275 ± 0.228	0.621 ± 0.313[a]	2.836 ± 0.129[ab]	2.177 ± 0.173[ab]	1.345 ± 0.142[ab]	1.246 ± 0.104[ab]
Estradiol (pmol/L)	264.145 ± 22.800	160.689 ± 1.682[a]	241.313 ± 11.849[b]	201.837 ± 1.679[ab]	179.738 ± 9.328[ab]‡	166.954 ± 27.264[a]
FSH (IU/L)	0.352 ± 0.048	0.208 ± 0.013[a]	0.313 ± 0.058[b]	0.324 ± 0.025[b]	0.207 ± 0.029[a]	0.222 ± 0.016[a]
LH (IU/L)	0.339 ± 0.022	0.182 ± 0.004[a]	0.323 ± 0.042[b]	0.326 ± 0.013[b]	0.190 ± 0.005[a]	0.179 ± 0.006[a]

Values are expressed as mean ± SD; n = 6 animals per group. [a]Adjusted p <.05 compared with G1; [b]adjusted p <.05 compared with G II. RV, R. vomitoria; AV, A. vogelii; IR, irradiation. ‡G V oestradiol was significantly different by the Games-Howell test but not by the rank-based sensitivity analysis and hence should be interpreted with caution.

Table 2. *Omnibus effects of treatment group on reproductive hormone concentrations*

Outcome	Primary test	Statistic	p	η^2	ω^2	Rank-based sensitivity
Testosterone	One-way ANOVA	F(5, 30) = 165.17	<.001	.965	.958	H(5) = 32.90; p < .001
Estradiol	Welch ANOVA	F(5, 13.05) = 337.36	<.001	.876 †	.852 †	H(5) = 30.46; p < .001
FSH	One-way ANOVA	F(5, 30) = 20.54	<.001	.774	.731	H(5) = 26.90; p < .001
LH	Welch ANOVA	F(5, 13.35) = 175.65	<.001	.940 †	.928 †	H(5) = 28.95; p < .001

Note: Holm adjustment resulted in no loss of significance for any of the primary omnibus effects. η^2, eta-squared; ω^2, omega-squared. †Classical variance-partition estimates are presented descriptively as Welch ANOVA was the primary test for estradiol and LH.

The treatment group exhibited significant effects on all reproductive hormones, with substantial effect sizes for testosterone (η^2 = .965), estradiol (η^2 = .876), FSH (η^2 = .774), and LH (η^2 = .940). These omnibus effects remained significant after Holm adjustment and were supported by rank-based sensitivity analyses (all p values < .001). Irradiation alone (G II) significantly reduced

testosterone, estradiol, FSH, and LH compared to the normal control (G1), indicating a marked disruption of reproductive endocrine function. In comparison to G II, the combined pre- and post-irradiation treatment regimens (G III and G IV) significantly increased all four hormones, with G III achieving the greatest recovery of testosterone and estradiol among the extract-treated groups. FSH and LH concentrations in G III and G IV were statistically similar to those in G1, while estradiol was restored to the control level only in G III. However, testosterone in G III remained significantly below G1, indicating substantial but incomplete recovery. Conversely, the pre-irradiation-only regimens (GV and G VI) increased testosterone but did not significantly improve FSH or LH; G VI also showed no benefit for estradiol. Although G V demonstrated a modest increase in estradiol under Games–Howell testing, this finding was not confirmed by the Dunn–Holm sensitivity analysis and should therefore be interpreted with caution. Overall, the findings suggest that treatment extending into the post-irradiation period resulted in broader endocrine recovery than pre-irradiation administration alone.

3.2 Pre- and post-irradiation administration versus pre-treatment alone

Direct within-extract comparisons revealed that G III exceeded G VI for testosterone, estradiol, FSH, and LH (adjusted $p < .001$, $p = .004$, $p = .001$, and $p = .002$, respectively). Similarly, G IV exceeded G V for testosterone, estradiol, FSH, and LH (adjusted $p < .001$, $p = .013$, $p < .001$, and $p < .001$, respectively). These comparisons indicated consistently higher hormone concentrations when administration continued after irradiation compared to when the corresponding extract was administered only before irradiation; however, the weaker contrasts should be interpreted with the sensitivity analyses.

Table 3. *Primary adjusted comparisons with the irradiation-only group*

Outcome	Comparison	Mean difference	95% simultaneous CI	Adjusted p
Testosterone	G III vs G II	2.215 nmol/L	1.916 to 2.513	<.001
	G IV vs G II	1.556 nmol/L	1.258 to 1.855	<.001
	G V vs G II	0.724 nmol/L	0.426 to 1.023	<.001
	G VI vs G II	0.625 nmol/L	0.327 to 0.924	<.001
Estradiol	G III vs G II	80.624 pmol/L	60.116 to 101.132	<.001
	G IV vs G II	41.148 pmol/L	37.779 to 44.518	<.001
	G V vs G II	19.049 pmol/L	2.957 to 35.140	.025†
	G VI vs G II	6.265 pmol/L	−41.157 to 53.687	.990
FSH	G III vs G II	0.105 IU/L	0.050 to 0.159	<.001
	G IV vs G II	0.115 IU/L	0.061 to 0.170	<.001
	G V vs G II	−0.001 IU/L	−0.056 to 0.053	>.999

Outcome	Comparison	Mean difference	95% simultaneous CI	Adjusted p
	G VI vs G II	0.013 IU/L	−0.041 to 0.068	.950
LH	G III vs G II	0.141 IU/L	0.069 to 0.213	.003
	G IV vs G II	0.144 IU/L	0.122 to 0.166	<.001
	G V vs G II	0.008 IU/L	−0.001 to 0.017	.105
	G VI vs G II	−0.003 IU/L	−0.014 to 0.008	.877

Note. Positive differences indicate higher concentrations than G II. Testosterone and FSH used Dunnett-adjusted confidence intervals; estradiol and LH used Games–Howell intervals. †Not confirmed by the rank-based sensitivity analysis.

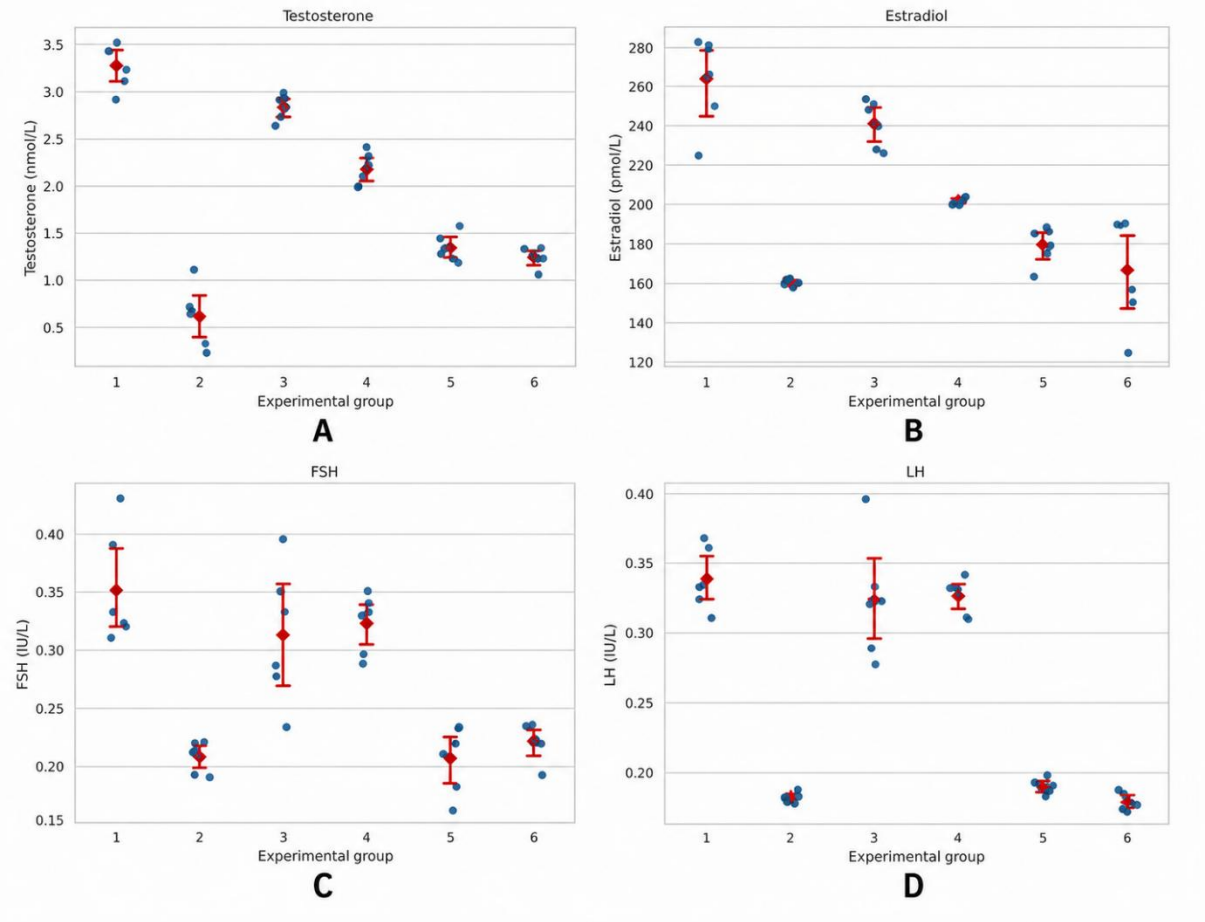

Figure 1 shown Serum reproductive hormone levels in the experimental group are. Blue dots represent individual animals (n = 6 per group) and red diamonds represent the group means with red error bars representing the 95% confidence intervals. Panels are arranged in the following order: testosterone (top left), oestradiol (top right), FSH (bottom left), LH (bottom right). The groups were designated as G1, normal control; G II, irradiation only; G III, *Rauwolfia vomitoria* given before and after irradiation; G IV, *Anthocleista vogelii* given before and after irradiation; G V, *Anthocleista vogelii* pre-treatment only; G VI, *Rauwolfia vomitoria* pre-treatment only.

3.3 Qualitative testicular histology

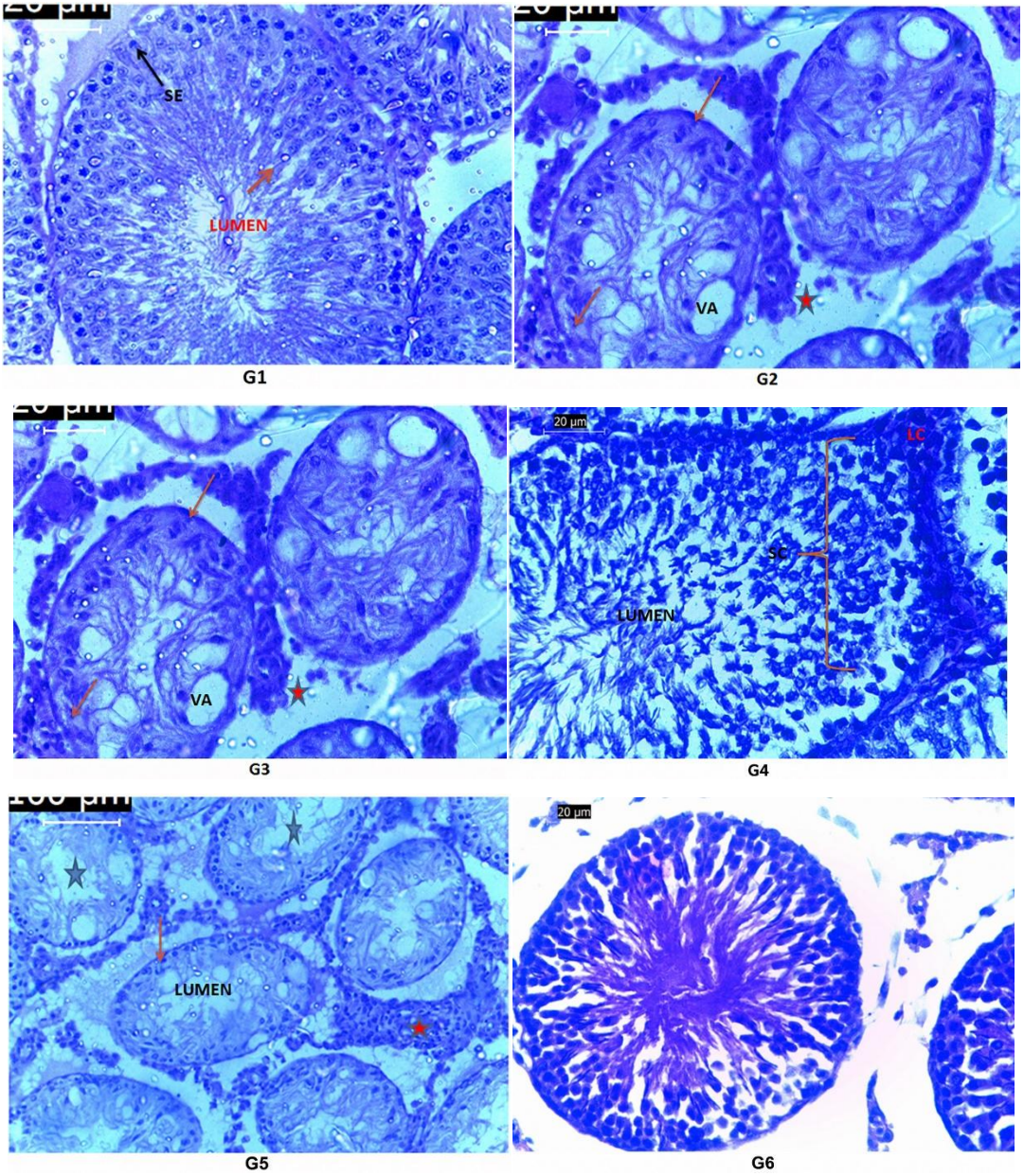

Figure 2 presents representative photomicrographs of H&E-stained testicular sections from the experimental groups. In Group 1 (G1) the architecture of the seminiferous-tubules is preserved with normal organization of the germinal epithelium. The black arrow indicates a Sertoli cell, the orange arrow an elongated spermatid, and LUMEN the seminiferous-tubule lumen containing spermatozoa. In Group II (G II) the peripheral orange arrow indicates basement-membrane thickening and the intratubular arrow reduced Sertoli-cell profiles. The red star indicates Leydig-cell hypoplasia, VA is germ-cell vacuolation; these changes are associated with severe tubular necrosis and the absence of spermatogenesis. Group III (G III) reveals preserved seminiferous tubules. Orange arrow points to a Sertoli cell and the red star indicates Leydig cells. SC stands for the spermatogenic-cell series and the black arrow points to luminal spermatozoa. In Group IV (G IV), the orange bracket labelled SC identifies the organised spermatogenic-cell series, LC denotes Leydig cells, and SP and LUMEN indicate spermatozoa and the tubular lumen, respectively. The orange

arrow indicates thickening of the basement membrane, the grey stars indicate severely atrophic, necrotic and degenerating seminiferous tubules and the red star indicates Leydig-cell hyperplasia in Group V (G V). Group VI (G VI) has relatively preserved seminiferous-tubule architecture, organized germinal epithelium and luminal spermatozoa.

4.0 DISCUSSION

The present study showed that a single 7.0-Gy abdominopelvic exposure caused significant and long-lasting testicular endocrine and structural damage in adult male Wistar rats. The irradiation only group (G II) showed a decrease of 81.0% in testosterone, 39.2% in oestradiol, 40.8% in FSH and 46.2% in LH after 8 weeks of irradiation as compared to the normal control. These biochemical changes were associated with basement membrane thickening, germ-cell vacuolation, Leydig-cell hypoplasia, severe tubular necrosis and absence of active spermatogenesis. The large effect sizes for testosterone (η^2 =.965), oestradiol (η^2 =.876), FSH (η^2 =.774), and LH (η^2 =.940) showed that the treatment group explained a large proportion of the variance observed in all four hormones. The main finding was that both extracts had an effect on reproductive indices and that treatment after irradiation was associated with a significantly greater endocrine recovery than with pre-treatment alone. Of the regimens tested *Rauwolfia vomitoria*before and after irradiation (GIII) gave the most consistent recovery in testosterone, oestradiol, FSH, LH and testicular architecture while *Anthocleista vogelii* before and after irradiation (GIV) resulted in a significant but less complete recovery of steroid hormones.

This marked suppression of testosterone in G II is consistent with the radiation-induced impairment of Leydig cell steroidogenesis and disruption of the cellular environment essential for androgen production. This is in line with clinical and experimental evidence showing that testicular irradiation can lead to Leydig cell dysfunction, reduced testosterone levels, and long-term reproductive endocrine dysfunction, although the extent of dysfunction is dependent upon dose, dose rate, age, and time after exposure (Baliga et al., 2024; Elenkov & Giwercman, 2022; Georgakopoulos et al., 2024; Spadella et al., 2026). The marked Leydig cell hypoplasia observed in G II provides a structural basis for the 81% reduction in serum testosterone and supports the interpretation that the hormonal deficit reflected true testicular injury rather than an isolated analytical fluctuation. This result further corroborates with direct rodent studies where ionizing radiation disrupted sex hormone balance, reduced testosterone-associated function, and produced oxidative, apoptotic, and histological testicular injury (Amer et al., 2022; Dil et al., 2023; Erdem et al., 2024; Farhadi et al., 2026).

Simultaneous decreases in LH, FSH, testosterone and oestradiol suggest a dysfunction not confined to isolated loss of Leydig cells. In simple primary testicular failure testosterone falls with a subsequent loss of negative feedback and an increase in gonadotropin secretion. Thus, the parallel decrease of LH and FSH seen in G II indicates a mixed disturbance of the hypothalamic–pituitary–gonadal axis, systemic radiation stress, or impaired communication between pituitary hormones and testicular somatic cells. Similar concurrent alterations in gonadotropins, testosterone, spermatogenesis and testicular morphology have been documented in experimental reproductive injury and have shown amelioration following antioxidant or anti-inflammatory interventions (Adana et al., 2022; Akhigbe et al., 2024; Amer et al., 2022; Seif et al., 2021). However, in the present study, we have not studied gonadotropin-releasing hormone, pituitary histology, steroidogenic enzymes, androgen receptors and aromatase. Therefore, it is impossible to identify the anatomical level and molecular basis of the endocrine disturbance from the present data.

The decline in oestradiol should be considered as a part of a more general steroidogenic disturbance and not as a single oestrogen problem. In men, oestradiol is produced mainly by aromatization of testosterone in testicular and peripheral tissues. Accordingly, the 81% decrease in testosterone and the damage to Leydig and Sertoli cells would probably decrease the cellular and substrate support for the

production of oestrogen. The present results of low testosterone, altered gonadotropins and impaired testicular morphology are in agreement with toxicological studies that indicated reproductive damage with simultaneous disturbance of several hormones and natural antioxidant interventions that ameliorated both endocrine and structural results (Akhigbe et al., 2024; Ogunlade et al., 2022; Seif et al., 2021; Srivastava et al., 2025). Because aromatase activity and intratesticular hormone levels were not measured, this explanation is biologically plausible but not experimentally proven.

The highest overall endocrine response was obtained by the *Rauwolfia vomitoria* regimen before and after irradiation. The levels of testosterone, oestradiol, FSH and LH in G III were increased by 2.215 nmol/L, 80.624 pmol/L, 0.105 IU/L and 0.141 IU/L respectively compared with G II. Oestradiol, FSH and LH levels were not significantly different from normal controls. Testosterone was still a bit low but considerably. This trend indicates a general but incomplete restoration of endocrine function. This is in agreement with reports that phytochemical-rich interventions ameliorate testicular steroidogenesis, modulate gonadotropins, boost antioxidant defences and preserve histological integrity following experimentally induced reproductive damage (Abarikwu et al., 2022; Akhigbe et al., 2024; El-Wafaey et al., 2025; Seif et al., 2021). This is consistent with investigations of radiation-specific interventions, showing that treatments that can ameliorate oxidative and inflammatory damage can preserve or restore testosterone-related function and spermatogenic architecture following exposure (Amer et al., 2022; Farhadi et al., 2026; Gezer & Karadag-Sari, 2022; Spadella et al., 2026). Reaction was essential before and after irradiation A. vogelii. G IV had significantly larger levels of all 4 hormones than GII, yet FSH and LH did not statistically vary from G1.

However, testosterone and oestradiol concentrations were lower than normal control values indicating that recovery of gonadotropins was more complete than the recovery of steroid hormones. This partial recovery is consistent with results from studies indicating that some natural products with antioxidant and anti-inflammatory properties are more effective than others in improving reproductive outcomes, as germ cell survival, somatic cell function, steroidogenesis and pituitary signalling do not recover at the same rates (Abarikwu et al., 2022; Akhigbe et al., 2024; Ali et al., 2023; El-Wafaey et al., 2025). These results are in line with the data of presence of flavonoids, polyphenols, alkaloids, saponins and other bioactive constituents in *Anthocleista vogelii* which may inhibit oxido-inflammatory mediators in experimental models (Aiwonegbe et al., 2025; Bassey et al., 2024; Nzor & Okoroh, 2025; Oredeko et al., 2023). G III exhibited a higher mean testosterone and oestradiol than G IV, however the data should not be construed as *Rauwolfia vomitoria* being innately more potent than A. vogelii. Preparation will vary with the plant species, the solvent used for extraction and the dose given. G III was treated with ethanolic extract of *R. vomitoria* (100 mg/kg) while G IV was treated with aqueous extract of *Anthocleista vogelii* (200 mg/kg). Furthermore, a pre-defined direct superiority comparison of G III with G IV was not performed in the study and the utilized extracts were not chemically standardized. Thus, the justifiable conclusion is that the preparation of *Rauwolfia vomitoria* studied offered a statistically greater recovery of steroid-hormone under the conditions used, and not that the species itself was demonstrated superior.

Qualitative histology corroborated well the biological efficiency of the irradiation paradigm. GII had thickening of the basement membrane, vacuolation, depletion of Sertoli cells, hypoplasia of Leydig cells, tubular necrosis and absence of active spermatogenesis. G1 had germinal epithelium with sperms in the lumen. This is consistent with the radiosensitivity of spermatogonia and other rapidly dividing germ cells and experimental descriptions of radiation induced tubular distortion, germ cell loss, vacuolation, impaired spermatogenesis and interstitial cell injury (Amer et al., 2022; Fukunaga et al., 2022; Georgakopoulos et al., 2024; Erdem et al., 2024; Zhi et al., 2025). Similar structural damage was attenuated by melatonin, amifostine, dexmedetomidine,

selenium nanoparticles and other interventions, thus validating the seminiferous architecture as a sensitive endpoint for testicular radioprotection (Amer et al., 2022; Dil et al., 2023; Farhadi et al., 2026; Gezer & Karadag-Sari, 2022). GIII and GIV showed comparatively well-preserved seminiferous tubules with orderly series of spermatogenic cells, Sertoli cells and Leydig cells and lumina spermatozoa. In these groups, histological preservation was associated with recovery of FSH and LH levels and significant increases in testosterone and oestradiol providing corroborative evidence of structural and functional recovery. The association between morphological and endocrine markers is in agreement with intervention studies where maintenance of testicular architecture was associated with improved testosterone levels, gonadotropin signaling, sperm characteristics, and antioxidant status and reduced inflammatory or apoptotic injury (Akhigbe et al., 2024; Ali et al., 2023; El-Wafaey et al., 2025; Seif et al., 2021). The data presented here support the idea that the efficacy of a testicular intervention should be judged by convergence of numerous complementing endpoints rather than by a single hormone or tissue field.

G V demonstrated the most severe failure of pre-treatment only protection with significant seminiferous tubule atrophy, necrosis, degeneration, basement membrane thickening, Leydig cell hyperplasia and sustained absence of spermatogenesis despite a minor increase in testosterone. Leydig cell hyperplasia may be a compensatory event in wounded interstitial tissue. However, in the absence of simultaneous LH recovery and by virtue of the qualitative nature of the judgement, no final mechanistic assertion can be made. This is a paradox which indicates that statistically increased quantities of testosterone do not necessarily mean that the spermatogenic compartment is preserved

The contrary trend was however noted in G VI. Its representative photomicrograph revealed intact tubules and luminal spermatozoa. However, oestradiol, FSH and LH levels were not different from G II whereas testosterone levels were substantially lower than G1. Structural and endocrine recovery may be at least largely dissociated, although this divergence may also be explained by field-selection effects, as histology was qualitative and based on representative images and not on blinded group-level morphometry. Quantitative measurements of seminiferous tubule diameter, epithelium height, Johnsen score, germ cell counts, Leydig cell density, and the percentage of aberrant tubules were needed to determine if the apparently preserved GVI field was representative of the group as a whole. The extracts are likely to have protective benefits due to their phytochemical and antioxidant capacities, however the proposed mechanisms were not investigated in this study. Alkaloids, flavonoids, saponins, tannins, glycosides and other compounds have been discovered from *Rauwolfia vomitoria* leaves. Ethanol or methanol extracts have exhibited antioxidant, radical scavenging, anti-inflammatory and membrane stabilizing activities (Ajayi, 2021; Kpadonou-Kpoviessi et al., 2024; Owoade et al., 2021; Ugwu et al., 2023). These properties may explain the high G III response, since radiation-induced reactive oxygen species, lipid peroxidation, inflammatory signaling, DNA damage and apoptosis are important factors in germ-cell and somatic-cell damage (Amer et al., 2022; Erdem et al., 2024; Mishra et al., 2025; Zhi et al., 2025). *Anthocleista vogelii* contains chemical compounds with antioxidant activity and extracts of this species have been shown to affect anti-inflammatory and oxido-inflammatory reactions (Aiwonegbe et al., 2025; Bassey et al., 2024; Nzor & Okoroh, 2025; Oredeko et al., 2023). The results obtained here support the partial endocrine and histological recovery in G IV and further demonstrate the capacity of flavonoid and polyphenol-rich interventions to protect testicular tissues through antioxidant, anti-inflammatory and anti-apoptotic mechanisms (Abarikwu et al., 2022; Akhigbe et al., 2024; El-Wafaey et al., 2025; Seif et al., 2021). However, the precise causative pathway could not be confirmed since the extracts were not identified and the measurements of testicular reactive oxygen species, MDA, GSH, SOD, catalase, cytokines, caspases, and DNA damage and steroidogenic proteins were not performed.

4.1 Strengths, Limitations and Implications for Translational Research

One of the major aspects of this work was the comparative study of two native medicinal plants with 4 reproductive hormones and testicular histology under the same irradiation circumstances. The study design included normal, irradiation only, pre-treatment only and pre plus post-treatment groups, to evaluate the protective and recovery-related effects of the treatments. Statistical robustness was further given by rank based sensitivity analyses, Holm correction, effect size estimations, confidence intervals and corrected post-hoc comparisons. The limited sample size may have introduced a bias and an overestimation of precision of the effect estimates. The histological evaluation was qualitative and did not report blinding, systematic field sampling, morphometry, and stereology and validated spermatogenic scoring. The lack of data on sperm characteristics, testis weight, copulation success and progeny also does not allow the interpretation of the intact histological architecture as recovered fertility. Furthermore, lack of unirradiated extract controls, chemically standardised preparations, various dosages, adequate solvents and mechanistic biomarkers makes it difficult to assess safety, reproducibility, direct comparison of potency and causal interpretation of the data. Single terminal measurement could not distinguish early radioprotection from delayed regeneration. Dose variability could be attributed to batch irradiation. Thus, these data remain preclinical with no evidence of human efficacy, safe dose, preserved fecundity or compatibility with cancer control (Baliga et al., 2024; Li et al., 2024; Su et al., 2025; Yumura et al., 2023). Future study should include standardised extract, quantified blinded histology, fertility end points, mechanistic biomarkers, toxicity and tumour prevention.

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