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Chronic low-dose natural radiation exposure and testicular response in wild *Rattus rattus* from high background radiation areas in Kerala, India

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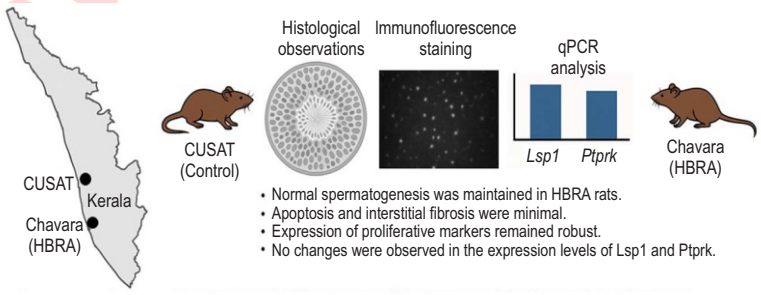
Abstract

Aim: Natural background radiation levels vary considerably worldwide; Chavara, located in Kerala, India, is known as a high-background radiation area (HBRA), where thorium-rich monazite sands produce radiation levels far exceeding the global average. The aim of this study was to evaluate the testicular histology and selected radiation-responsive gene expressionin wild male rats (*Rattus rattus*). Individuals captured from the Chavara HBRA and a nearby low-radiation area around the Cochin University of Science and Technology (CUSAT) were compared.

Methodology: Testicular tissues were examined by histological observations, immunofluorescence staining, and qPCR analysis of radiation-responsive genes (*Lsp1, Ptprk*) to evaluate spermatogenesis, apoptosis, and gene expression.



Chronic natural radiation was not associated with detectable impairment of spermatogenic parameters in *Rattus rattus.*

Results: Histological observations revealed preserved seminiferous tubule architecture and active spermatogenesis in HBRA individuals. PCNA expression indicated sustained proliferative activity, and no differences were detected in Lsp1 or Ptprk expression between groups. Apoptotic cells were less frequent in HBRA individuals; however, the overall testicular structure remained comparable between the regions.

Interpretation: Within the limitations of this field-based study, chronic natural radiation exposure was not associated with detectable impairment of spermatogenic parameters in wild *R. rattus*. However, subtle molecular or epigenetic effects cannot be excluded. Further investigations incorporating broader molecular analyses and larger sample sizes are warranted to clarify long-term biological consequences.

Key words: Environmental radiation, Low-dose exposure, *Rattus rattus,* Spermatogenesis

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Introduction

Humans and wildlife are continuously exposed to natural background radiation that originates from cosmic rays and terrestrial radionuclides. In most environments, radiation levels are low and generally considered to pose minimal biological risk. However, certain geological settings such as Ramsar (Iran), Yangjiang (China), and Chavara (India) exhibit markedly elevated levels of naturally occurring ionising radiation. These regions, designated as high background radiation areas (HBRAs), often display ambient dose rates more than ten times the global average (Nair *et al.,* 2009; Wei *et al.,* 1990). Chavara, in the South-western India, is characterised by monazite-rich coastal sands that contain high concentrations of thorium and uranium. The annual background radiation dose in this area can reach 15m Sv, approximately 30 times the Japanese average. While doses below 100 mSv are generally categorised as "low dose" by the International Commission on Radiological Protection (ICRP, 2007) and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2000), the long-term biological consequences of chronic low-dose exposure under natural environmental conditions remain incompletely understood (UNSCEAR, 2012). The radiation levels in Chavara were not experimentally selected but reflect naturally occurring geological conditions associated with thorium-rich monazite deposits.

This region has been recognized internationally as a representative high background radiation area and therefore provides a suitable natural model for examining long-term, low-dose exposure under environmental conditions. The male reproductive system is considered highly radiosensitive, and many experimental and epidemiological studies have documented disruptions in spermatogenesis and testicular architecture following exposure to ionising radiation (Bonisoli-Alquati *et al.,* 2011; Yin *et al.,* 2025). In addition to histological alterations, recent studies have demonstrated that low-dose ionizing radiation can induce subtle molecular and immunological changes in the testicular microenvironment, including oxidative stress responses and epigenetic modifications that may affect the sperm quality and fertility (Yin *et al.,* 2025). However, radiation responses may differ substantially among species and can be influenced by ecological context, evolutionary history, and life-history traits. Therefore, it is not appropriate to directly apply findings obtained from one species to another without sufficient empirical validation (Møller and Mousseau, 2015; Mothersill and Seymour, 2001). Previous studies conducted in the Fukushima-affected region around the Nuclear Power Plant, where environmental radiation levels were comparable to those observed in Chavara, reported no overt morphological abnormalities in wild rodents.

These studies reported no overt morphological abnormalities in the reproductive function. However, transcriptomic changes have been detected, suggesting that even low-dose environmental radiation can elicit molecular-level biological responses (Tokita *et al.,* 2025). Lymphocyte-specific 1 (*Lsp1*) and protein tyrosine phosphatase receptor type K (*Ptprk*) have been identified as candidate radiation-sensitive biomarkers in large Japanese field mice (*Apodemus speciosus*). Additional studies have shown that chronic radiation exposure in rodents is associated with changes in spermatogenic activity and a reduction in apoptosis (Takino *et al.,* 2017). Although chronic radiation exposure has been reported to affect the reproductive systems of certain species, its long-term effects under natural environmental conditions remain insufficiently understood. Moreover, emerging evidence suggests that radiation exposure may exert multi- and transgenerational effects in wildlife species, highlighting the complexity of long-term biological responses under environmental conditions (Sreetharan *et al.,* 2024). In view of the above, the present study aimed to examine the testicular histology, apoptosis frequency, and the expression of selected radiation-responsive genes (*Lsp1* and *Ptprk*) in wild *Rattus rattus* inhabiting a high background radiation area.

Materials and Methods

Field sampling and subsequent analyses of testicular histology and germ cell-specific gene expression were conducted in animals collected from high background radiation areas (HBRA) and control regions to evaluate the effects of chronic natural radiation exposure on spermatogenic parameters in wild rats *R. rattus*). Only individuals with histologically confirmed active spermatogenesis were included in the analysis to minimize potential bias arising from seasonal reproductive variation or the presence of inactive testes.

Study sites and Field sampling: The high-radiation site was Chavara, located in Southern Kerala, India (Fig. 1A), where coastal sands contain high concentrations of monazite, a thorium- and uranium-rich mineral. The vicinity of the Cochin University of Science and Technology (CUSAT) Marine Science Campus, located approximately 150 km north of Chavara, was selected as the control site. The study was conducted at CUSAT (9.964° N, 76.283° E), Site 1 (8.996° N, 76.523° E), and Site 2 (8.966° N, 76.530° E). The field surveys were conducted in October 2023 (Chavara) and November 2023 (CUSAT). Sherman live traps baited with taro and fried bread were set near the farmland and residential zones and checked the following morning. The captured animals were humanely euthanized by cervical dislocation in accordance with institutional ethical guidelines and both testes were excised (Table 2). Karyotypic analysis of bone marrow cells from the captured wild rodent confirmed that it was *R. rattus*. Gamma-ray spectra at the capture sites were measured 1m above the ground using the GPS-linked radiation monitoring system, Bio-Safety Hybrid Automatic Monitor-Niigata (BISHAMON) (Goto *et al.,* 2016) equipped with a CsI detector (Hamamatsu Photonics C12137-01, Shizuoka, Japan). Ambient dose equivalent rates H*(10) were calculated using the G(E) function method (Tanigaki *et al.,* 2015; Tsuda *et al.,* 2012) from the gamma-ray spectra. Ambient gamma radiation was directly measured on site by the authors using the calibrated BISHAMON system to ensure accurate assessment of environmental

radiation levels.

Histological analysis: One testis from each individual was fixed in 10% paraformaldehyde in phosphate-buffered saline (PFA), embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H&E). Histological evaluation was performed using a light microscope to assess spermatogenesis within the seminiferous tubules (Takino *et al.,* 2017). Ten tubules at stages VII–VIII were randomly selected per individual, and the number of total spermatogenic cells and elongating spermatids per tubule was counted to obtain mean values.

ApopTag Plus Peroxidase in situ: Apoptotic cells in *R. rattus* testes were detected using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Millipore, Temecula, CA, USA) according to the manufacturer's instructions. Briefly, paraffin-embedded testicular sections were deparaffinized and rehydrated, followed by antigen retrieval in 10 mM citrate buffer (pH 6.1). Endogenous peroxidase activity was blocked prior to incubation with terminal deoxynucleotidyl transferase (TdT) enzyme. After incubation, sections were treated with anti-digoxigenin peroxidase and visualized using diaminobenzidine (DAB) as the chromogen. Sections were counterstained, dehydrated, and mounted. Images were acquired using a LeitzLaborlux S microscope equipped with an Olympus digital camera.

Heidenhain's AZAN trichrome staining: Paraffin-embedded sections of formalin-fixed testis tissue were de-paraffinized, hydrated using xylene and a graded alcohol series, and processed for trichrome staining (Electron Microscopy Sciences, Danvers, MA, USA), according to the manufacturer's instructions.

Immunofluorescence staining: Immunofluorescence staining was performed to evaluate proliferative activity using a primary antibody against proliferating cell nuclear antigen (PCNA; ab2426, Abcam, USA) (Takino *et al.,* 2017). Formalin-fixed, paraffin-embedded sections were deparaffinized and rehydrated using standard protocols. Antigen retrieval was performed in Hist VT One solution (NacalaiTesque, Japan) at 90°C for 30 min. After blocking with 5% bovine serum albumin (BSA), sections were incubated overnight at 4°C with anti-PCNA antibody (1:200 dilution). Following washing, sections were incubated with Alexa Fluor 488–conjugated anti-rabbit IgG secondary antibody (ab150073, Abcam; 1:400 dilution) for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI (1:200), and sections were mounted using Fluoromount. Fluorescence images were captured using an EVOS M5000 Imaging System (Thermo Fisher Scientific, USA).

Gene expression analysis: The contralateral testis was immersed in RNA stabilization solution (RNAlater) and stored at −80 °C until processing. Total RNA was extracted using TRIzol Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA quality and concentration were assessed using a NanoDrop One spectrophotometer, and only samples with a 260/280 absorbance ratio between 1.8 and 2.0 were used for further analysis. Complementary DNA (cDNA) was synthesized from 0.8 µg of total RNA using the PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio, Japan). Quantitative PCR (qPCR) was performed using SYBR Premix Ex Taq II (Takara Bio) on a Thermal Cycler Dice® Real Time System under standard cycling conditions. Target genes included *Lsp1* and *Ptprk,* previously reported as radiation-responsive biomarkers in wild rodentsby Tokita *et al.,* 2025. Primers were redesigned based on previous *Apodemus speciosus* studies and optimized for R. rattus using Primer-BLAST.

Lsp1 gene:
Forward primer: 5'-ATGGCTGTAGCCAGTACCAA-3'
Reverse primer: 5'-GGGGTGCTCTTGATGGTGGA-3'
Ptprk gene:
Forward primer: 5'-GGCAACCAGCAGCTTTCATC-3'
Reverse primer: 5'-CAGCCCTGGGACAAGTCCAC-3'

PCR cycling conditions consisted of an initial denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and annealing/extension at 60°C for 30s. Amplification specificity was confirmed by melting curve analysis. Gene expression levels were normalized to the housekeeping gene β-actin and calculated using the 2^–ΔΔCt method. Only individuals with histologically confirmed active spermatogenesis were included in the qPCR dataset to ensure analytical consistency.

Statistical analysis: As this study was conducted under field conditions involving wild animals, the number of captured individuals was limited. In addition, only a small number of individuals exhibited active spermatogenesis (n = 1 in the control group and n = 2 in the HBRA group). Accordingly, statistical interpretation was regarded as limited and exploratory rather than confirmatory.

Results and Discussion

Ambient radiation dose rates in Chavara ranged from 0.09 to 16.5 µSv h^{-1}, with a mean of 1.7 µSv h^{-1}, corresponding to an estimated annual dose of approximately 15 mSv (Fig. 1A, B). This level was broadly comparable to those reported in the areas affected by the Fukushima Daiichi Nuclear Power Plant accident several months after the event, excluding highly contaminated zones later designated as difficult-to-return areas NRA, 2011). In contrast, the control site near the CUSAT campus exhibited dose rates ranging from 0.05 to 0.14 µSv h^{-1}, with a mean of 0.1 µSv h^{-1}, corresponding to an annual dose of approximately 0.9 mSv. These results confirm a clear difference in environmental radiation levels between the study sites. Within this exposure range, chronic exposure to elevated natural background radiation was not associated with measurable impairment of spermatogenic histological or molecular parameters in wild *R. rattus* inhabiting high-background radiation area (HBRA) of Chavara, Kerala, India. Histological examination of H&E-stained testicular sections from HBRA individuals (n=2) revealed

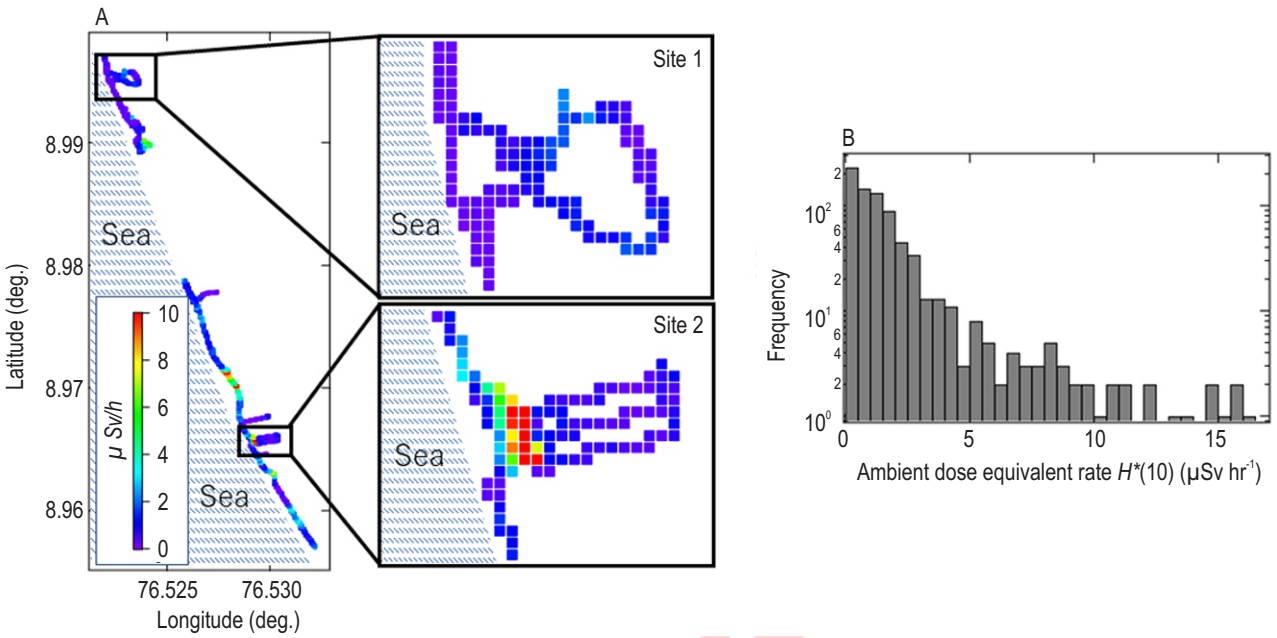

Fig. 1: Geographic location of the study sites and radiation dose distribution. (A) Spatial distribution of ambient gamma dose rates (measured in μSv hr^{-1}) in the Chavara coastal area, obtained using the BISHAMON system with integrated GPS mapping. The dose rates shown on the map represent values averaged over 10 m × 10m grid cells. Enlarged maps of the two sites used for environmental monitoring and animal trapping are also shown. (B) Histogram of dose rates for each 10 m × 10 m grid cell in the Chavara area. The minimum, maximum, and mean dose rates are 0.09 μSv hr^{-1}, 16.5 μSv hr^{-1}, and 1.7 μSv hr^{-1}, respectively.

seminiferous tubule architecture consistent with active spermatogenesis (Fig. 2). The seminiferous tubules were clearly delineated and exhibited a stratified germinal epithelium containing spermatogonia, primary and secondary spermatocytes, and spermatids. Elongating spermatids and spermatozoa-like structures were observed in some tubules. Interstitial tissue contained eosinophilic cells morphologically consistent with Leydig cells. In contrast, reduced germinal epithelium and decreased spermatogenic activity were observed in some control individuals (Nos. 9 and 11), likely reflecting seasonal variation; therefore, these individuals were excluded from subsequent analyses. Only individuals with histologically confirmed active spermatogenesis were included in gene expression analysis. Quantitative analysis revealed no differences in the total number of spermatogenic cells or elongated spermatids per seminiferous tubule between the HBRA group (Nos. 1 and 5) and the control group (No. 12) (Fig. 3A, B). Although the average number of spermatogenic cells tended to be slightly higher in the HBRA group, this difference was not significant. These findings indicate that chronic radiation exposure was not associated with measurable changes in spermatogenic cell populations.

Apoptosis detection using the ApopTag assay showed that apoptotic cells were widely distributed across multiple layers of the seminiferous epithelium in the control group. In contrast, in the HBRA group, apoptotic cells were sparse and primarily restricted to the spermatogonial layer adjacent to the basal lamina (Fig. 4). Because germ cells are highly radiosensitive (Dewey *et al.,* 1995), this reduced frequency of apoptosis may reflect adaptive regulation of cell death pathways under chronic exposure conditions (Liu *et al.,* 2006). Heidenhain's Azan trichrome staining further confirmed well-preserved seminiferous tubule architecture and active spermatogenesis in both groups (Figs. 5,6). The interstitial tissue contained numerous eosinophilic Leydig cells, and vascular structures were clearly visible. Collagen fibres were present within physiological limits, with no evidence of interstitial fibrosis. These findings support the absence of detectable structural damage associated with chronic radiation exposure. Leydig and Sertoli cells appeared morphologically intact, consistent with their relative radioresistance (Mettler, 2012). Mild stromal hyperplasia without fibrosis was observed, suggesting that any adaptive inflammatory response remained controlled. In contrast, more pronounced interstitial changes were observed in control individuals, raising the possibility that chronic radiation exposure may activate regulatory mechanisms that contribute to tissue homeostasis (Nguyen-Thanh *et al.,* 2022).

Immunofluorescence analysis using PCNA as a marker of cell proliferation demonstrated strong nuclear signals in both HBRA and control groups (Fig. 7). PCNA-positive cells were distributed across multiple layers of the germinal epithelium, particularly in the spermatogonia and primary spermatocytes,

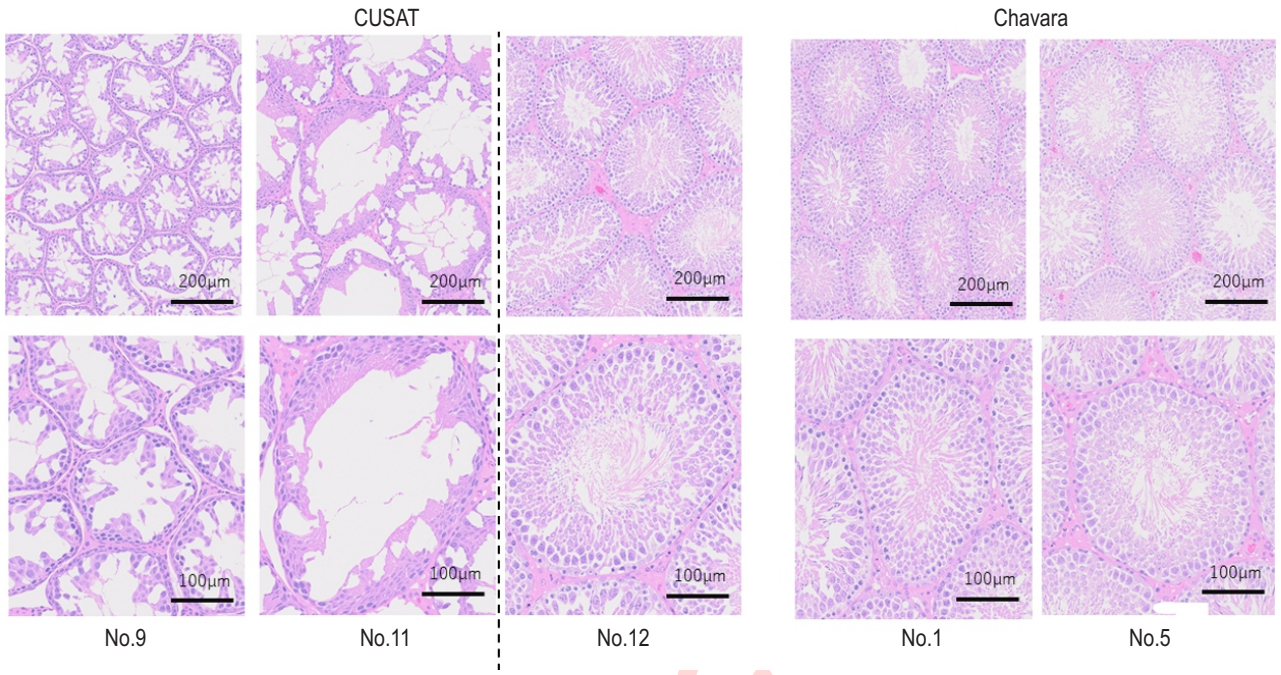

Fig. 2: Representative testicular histology of wild *Rattus rattus* from CUSAT (control) and Chavara (HBRA) regions. Hematoxylin and eosin (H&E) stained sections of seminiferous tubules from control individuals (left, CUSAT region: No. 9, No. 11, No. 12;n = 3) and HBRA-exposed individuals (right, Chavara region: No. 1, No. 5; n = 2). Upper panels show low magnification views (scale bar = 200 µm); lower panels show higher magnification of the same tubules (scale bar = 100 µm). Normal spermatogenic progression was observed in both groups, with layered germ cell development evident in HBRA individuals.

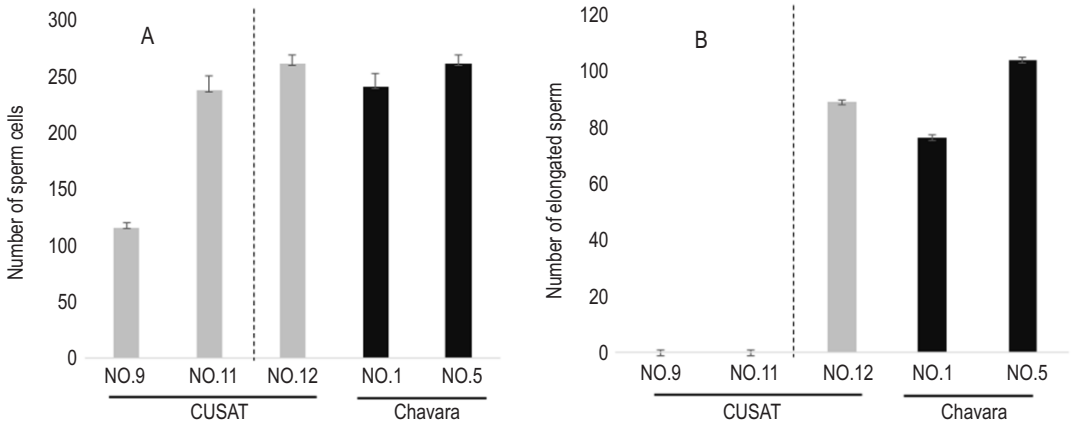

Fig. 3: Comparison of germ cell counts and elongating spermatid counts per seminiferous tubule. (A) Average number of total spermatogenic cells per seminiferous tubule in *Rattus rattus* individuals from the control (CUSAT region: No. 9, No. 11, No. 12;n = 3) and HBRA (Chavararegion: No. 1, No. 5; n = 2). (B) Average number of elongating spermatids per tubule in the same individuals. Data are presented as mean ±S.E., with values shown for each animal (CUSAT region: No. 9, No. 11, No. 12; n = 3 and Chavara region: No. 1, No. 5; n = 2).

indicating active DNA replication and robust proliferative activity. No apparent differences in staining intensity or distribution were observed between groups. These findings are consistent with the preservation of normal spermatogenic organisation and may reflect activation of protective cellular mechanisms such as enhanced DNA repair or antioxidant defence (Calabrese and Baldwin, 2002; Lau *et al.,* 2021; Luckey, 2006). Quantitative PCR analysis revealed no differences in the expression levels of the radiation-responsive genes *Lsp1* and Ptprk between the HBRA and control groups (Fig. 8). The relative expression of *Lsp1*

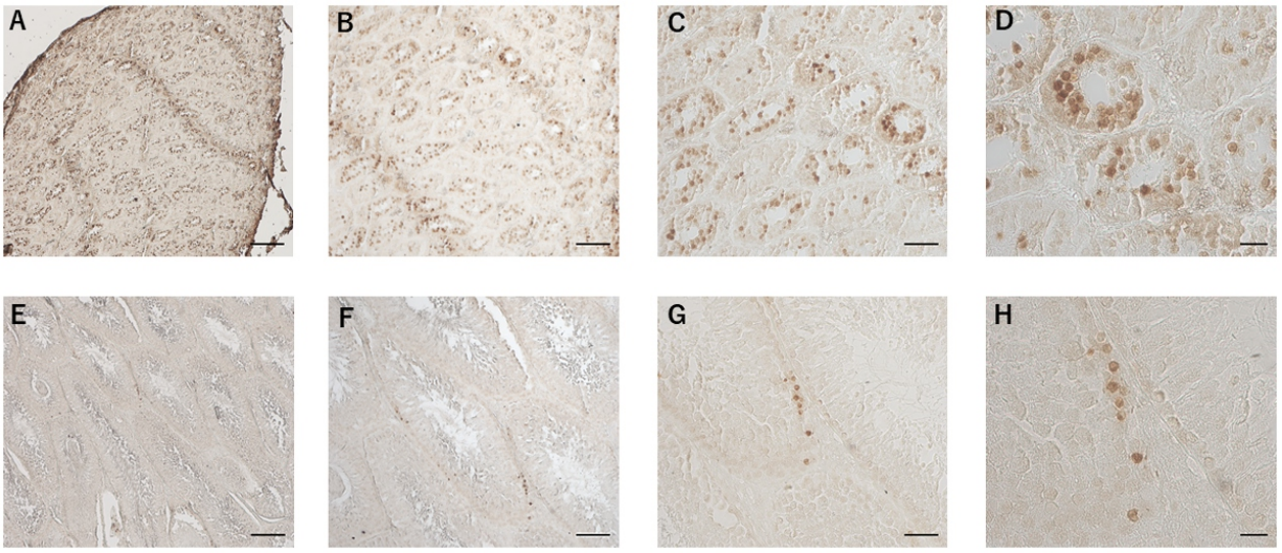

Fig. 4: Detection of apoptotic cells in the testes of *Rattus rattus* using the ApopTag Plus Peroxidase in situ apoptosis detection method. (A–D) Testicular sections from the control group (CUSAT region: No. 9, No. 11, No. 12; n = 3). (E–H) Testicular sections from the Chavara high-background radiation area (Chavararegion: No. 1, No. 5; n = 2). (A, E) Low-magnification light micrographs; (B–D, F–H) high-magnification views. Magnifications: A, E = 5× (scale bar = 200 µm); B, F = 10× (scale bar = 100 µm); C, G = 20× (scale bar = 50 µm); D, H = 40× (scale bar = 20 µm).

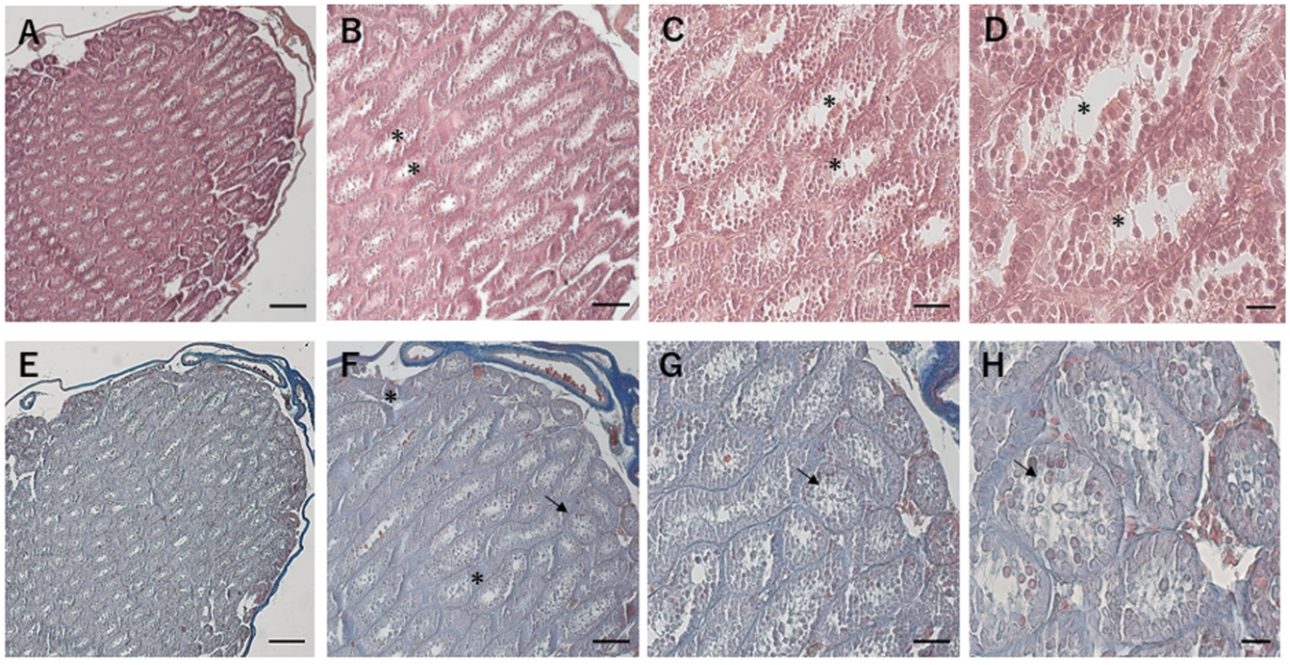

Fig. 5: Histological structure of *Rattus rattus* testis collected from the Chavara high-background radiation area (HBRA). Hematoxylin and eosin (H&E; A–D) and Heidenhain's Trichrome AZAN (E–H) staining of testicular tissue. (A, E) Low-magnification light micrographs; (B–D, F–H) high-magnification views. Magnifications: A, E = 5× (scale bar = 200 µm); B, F = 10× (scale bar = 100 µm); C, G = 20× (scale bar = 50 µm); D, H = 40× (scale bar = 20 µm). In (A), indicates the disorganisation and morphological alteration of germ cells. In (B), the asterisk (*) denotes hyperproliferation of interstitial cells, and the arrowhead shows loosened adhesion between spermatogonia and the basal lamina. Panels (C) and (D) show mature spermatozoa within the lumen. In (E), indicates the absence of the tubular lumen. In (F), the asterisk (*) indicates intratubular vacuolisation, whereas in (G) it indicates interstitial vacuolisation. In (G), the arrowhead denotes immature germ cells detached from the lumen, whereas in (H), the arrowhead indicates immature spermatozoa within the lumen.

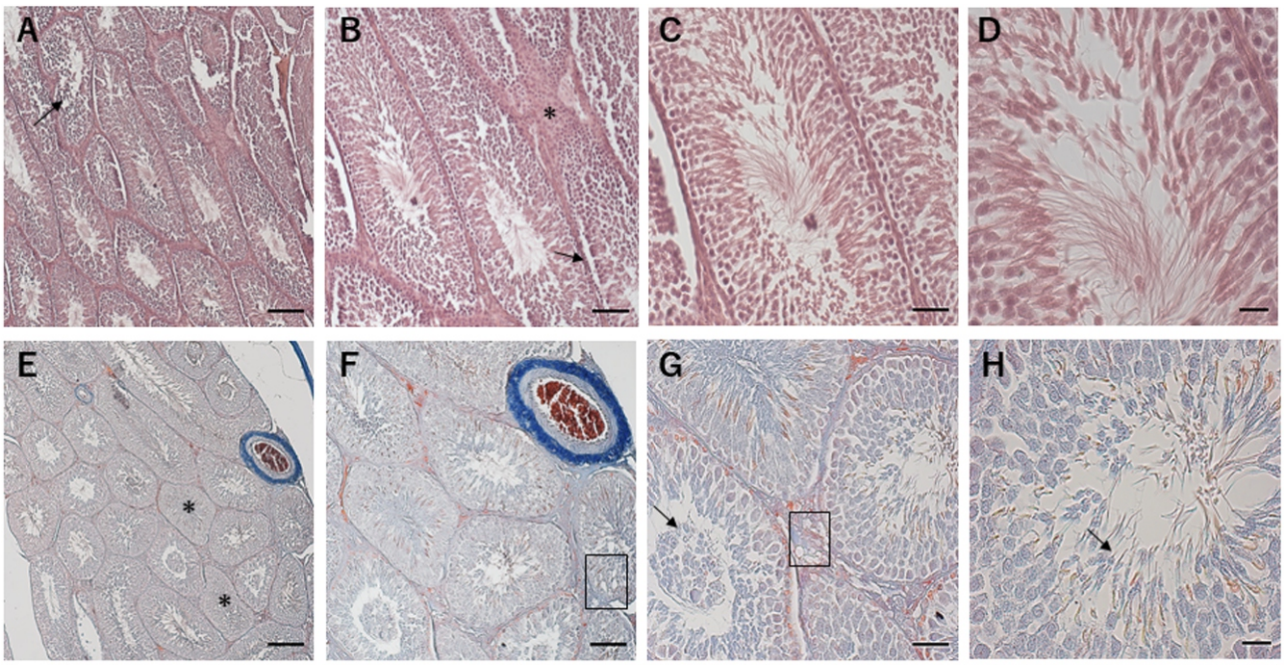

Fig. 6: Histological features of *Rattus ratrus* testis collected from the Chavara region. Hematoxylin and eosin (H&E; A–D) and Trichrome AZAN (E–H) staining of testicular tissue. (A, E) Low-magnification light micrographs; (B–D, F–H) high-magnification views. Magnifications: A, E = 5× (bar = 200 µm); B, F = 10× (bar = 100 µm); C, G = 20× (bar = 50 µm); D, H = 40× (bar = 20 µm). In panel A, the arrowhead indicates the disorganisation and morphological alteration of germ cells. In B, the asterisk (*) denotes hyperproliferation of interstitial cells, and the arrowhead shows loosened adhesion between the spermatogonia and the basal lamina. Panels C and D reveal the presence of mature spermatozoa within the seminiferous tubule lumen. In E, the asterisk marks the absence of a tubular lumen. In F, the boxed region indicates intratubular vacuolisation, whereas in G, the boxed region indicates interstitial vacuolisation. In G, the arrowhead denotes immature germ cells detached from the lumen; in H, the arrowhead indicates mature spermatozoa within the lumen.

Table 1: Individual characteristics of captured wild *Rattus rattus*

Region	Number of rates	Sampling date	Body weight (g)	Body length (cm)	Testis weight (g)	Testis length (mm)
Chavara	1	2023/10/20	96.0	143.4	0.67	17
	5	2023/10/21	144.9	193.4	1.03	20
CUSAT	9	2023/10/31	57.2	134.0	0.21	10
	11	2023/11/03	115.3	170.0	1.2	19
	12	2023/11/03	90.2	152.0	1.12	19

Summary of biometric and reproductive parameters for each animal captured in the Chavara (HBRA) and CUSAT (control) regions. Mitochondrial COI gene sequencing confirmed that all individuals belonged to *Rattus rattus*.

averaged 1.39 in the HBRA group and 1.25 in the control group, whereas Ptprk expression averaged 1.22 and 1.00, respectively. Both the genes showed a slight upward trend in the HBRA group, but no clear radiation-associated effect was detected. Together, histological and molecular analyses revealed well-preserved testicular architecture, active spermatogenesis, and stable expression of selected radiation-responsive genes.

These findings indicate that chronic low-dose radiation exposure in high background areas was not associated with detectable disruption of spermatogenic parameters in this species. However, it should be noted that in this study histological features and selected molecular markers were evaluated, but did not directly assess sperm count, motility, or fertility. Therefore, the conclusions are limited to spermatogenic tissue-level parameters rather than overall reproductive performance. The absence of detectable impairment may reflect the capacity of testicular tissue to maintain homeostasis under prolonged low-dose exposure through coordinated DNA repair, antioxidant defence, and regulated germ cell turnover. Consistent with this interpretation, apoptosis was less frequent in HBRA individuals, suggesting a shift in the regulation of cell death pathways. In particular, the absence of detectable histological or gene expression differences does not exclude subtle molecular, epigenetic, or

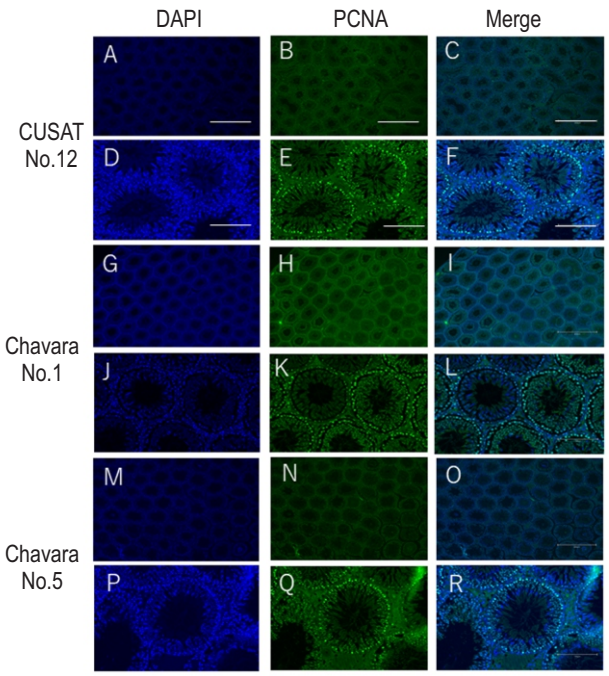

Fig. 7: Double immunofluorescence staining of PCNA and DAPI in Rattus rattus testes collected from the high-background radiation area (Chavara) and the control region (CUSAT). Panels A–R show merged images of DAPI (blue) and PCNA (green) signals in seminiferous tubules of testicular sections. Panels A–F represent images from the same control individual (No.12) shown at different magnifications (4× and 20×). Panels G–L and M–R represent two HBRA individuals (No.1 and No.5), each shown at corresponding low and high magnifications. Scale bar = 100 µm.

transgenerational effects beyond the scope of this study. Previous studies have reported that low-dose radiation may induce transcriptomic, epigenetic, or immune-related alterations that are not readily detectable by conventional histological approaches (Yin *et al.,* 2025). The findings of this study is in contrast with the previous observations in Apodemus speciosus inhabiting Fukushima-affected areas, where significant alterations in *Lsp1* and *Ptprk* expression were detected under comparable radiation levels (Tokita *et al.,* 2025). This discrepancy emphasises the species-specific nature of radiation responses. Species-dependent variability in radiation sensitivity has been documented across multiple taxa, suggesting that ecological and evolutionary factors play critical roles in shaping radiation responses (Sreetharan *et al.,* 2024). Differences in taxonomy, metabolism, life-history strategy, and ecological adaptability between *R. rattus* and *A. speciosus* make it unlikely that identical biomarkers function consistently across taxa. Accordingly, validation of radiation-sensitive markers should be conducted in a species- and context-dependent manner (Møller and Mousseau, 2015; Mothersill and Seymour, 2001).

It is plausible that *R. rattus* populations in Chavara have experienced elevated radiation exposure over many generations, potentially promoting physiological or genetic adaptations. Comparable adaptive mechanisms have been proposed in organisms inhabiting chronically irradiated environments, such as the Chernobyl region (Esnault *et al.,* 2010; Webster *et al.,* 2016). Such long-term, field-based exposure differs fundamentally from acute laboratory conditions and underscores the ecological and evolutionary relevance of field studies in radiation biology (Deryabina *et al.,* 2015; Møller and Mousseau,

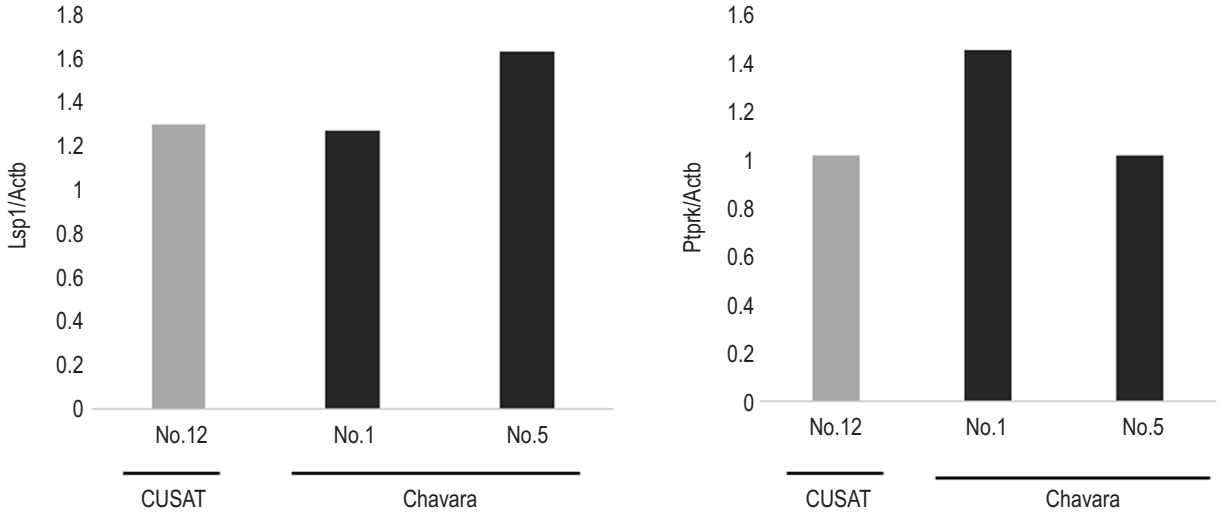

Fig. 8: Relative expression levels of *Lsp1* and *Ptprk* genes in *Rattus rattus* testes as measured by qPCR. Left: Relative expression of *Lsp1* normalised to *Actb* (β-actin) in individuals from the control region (CUSAT region: No.12; n =1) and high-background radiation area (Chavara region: No.1 and No.5; n =2). Right: Relative expression of Ptprk in the same individuals. Gene expression was quantified using the 2^–ΔΔCt method.

2006).Although this study provides novel field-based insights, the sample size was limited and confined to a single season. In addition, potential biological confounders, including seasonal reproductive variation, age differences, and individual variability, could not be fully controlled under field conditions. These factors should be considered when interpreting the findings.

In conclusion, wild *R. rattus* inhabiting the Chavara region exhibited preserved spermatogenic structure and stable expression of selected radiation-responsive genes under chronic natural radiation exposure within the limits of this study. Although no detectable impairment of spermatogenesis was observed, these findings should be interpreted with caution. The absence of detectable alterations should not be interpreted as definitive evidence of the absence of biological effects. Further investigations incorporating larger sample sizes, multi-season sampling, and comprehensive molecular analyses are required to fully elucidate the long-term biological consequences of environmental radiation exposure.

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