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UNIVERSITY GRANTS COMMISSION, NEW DELHI

STUDY OF THE EFFECT OF NATURAL BACKGROUND RADIATION ON CERTAIN RODENTS (*IN VITRO*) – A CYTOGENETIC APPROACH

[F. No. – 43-555/2014(SR) Dated 29 Dec, 2015]

SCOTT CHRISTIAN COLLEGE (Autonomous)

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CHAPTER I
INTRODUCTION

1. INTRODUCTION

Everyone is exposed to natural radiation. Radiation represents electromagnetic waves and particles that can ionize, that is, remove an electron from an atom or molecule of the medium through which they propagate. The main sources of radiation include cosmic rays and naturally occurring radionuclides present in the earth's crust, in building materials and in air, water and foods and in the human body itself. More than 60 radionuclides can be found in the environment, which can be divided into three general categories: primordial, cosmogenic, and manmade. A significant amount of natural radiation is due to radon gas (^{222}Rn), which is released from the soil and may concentrate in dwellings. Large amount of radionuclides such as ^{232}Th, ^{238}U, ^{226}Ra, ^{40}K, etc., are found accumulated in soil and rocks in some parts of the world (Ramsar in Iran, Yangjiang in China, Kerala in India, Guarapari in Brazil, etc.), with considerably high level of radiation.

These radionuclides produce an external radiation field due to natural decay of some unstable nuclei to which human beings are exposed. Some of the exposures are fairly constant and uniform for all individuals everywhere, for example, the dose from ingestion of ^{40}K in foods. Other exposures like cosmic rays and background radiation vary widely depending on location. Cosmic rays, for example, are more intense at higher altitudes, and background radiation is elevated in certain areas due to high concentrations of uranium and thorium in soils (UNSCEAR, 2000).

These radiations are capable of altering the structure and function of the cell, which leads to cellular damage. The damage may result in cell death or modifications that can affect the normal functioning of organs and tissues. But most organs and tissues of the body are not affected by the loss of even considerable numbers of cells.

Cytogenetic analyses have been used as an important biological tool to study the mutagenic effects of ionizing radiation and are some of the few methods available for screening alterations to the entire genome of individual cells. Chromosomal aberrations (CAs) usually occur when there is an error in cell division following meiosis or mitosis and have been correlated with genetic changes that can trigger the development of cancer. Therefore, a biological dosimeter based on CA frequencies makes it possible to estimate the cancer risk.

Radiation-exposed human populations have been most extensively studied by using fluorescent *in situ* hybridisation (FISH); but only a few chromosomes can be examined by using two- or three-colour FISH to detect chromosomal translocations (Tucker, 2001). This is difficult for the study of complex CAs, which involves multiple chromosomes. The development of multifluor FISH (mFISH) (Speicher *et al*., 1996), in which all 22 autosomes plus the two sex chromosomes are painted in different colours to determine chromosome alterations, has greatly improved our ability to identify CAs in general and complex aberrations in particular.

Analysis of micronuclei (MN) in cytokinesis-blocked micronuclei assay (CBMN) is an easy and quick procedure that has been suggested as an alternative method to detect chromosomal alterations induced by ionizing radiations. MN

induction in human lymphocytes has proved to be a sensitive method for assessment of cytogenetic damage induced by ionizing radiation (Fenech and Morley, 1985).

DNA damage in the individual cell can be assessed by using comet assay, or single-cell gel-electrophoresis assay (SCGE). The damaged cellular DNA containing fragments and strand breaks is separated from the intact DNA, yielding a classic comet-tail shape under the microscope. The extent of DNA damage can be calculated by usually measuring the length of the comet tail.

Cells have a number of DNA repair systems that can correct many forms of DNA damage that have been induced spontaneously or by external agents. The induction and development of cancer after radiation exposure is not the stepwise accumulation of mutations in the DNA of the relevant cells. There have been studies relating to the following hypotheses: (a) that adaptation of cells and tissues to low doses of radiation might cause them to become more resistant to cancer development (adaptive response); (b) that the effects of radiation on the immune systems, which recognize and destroy abnormal cells, could influence the likelihood of cancer development; and (c) that radiation can produce changes that create long-lasting and transmissible effects on the stability of cellular DNA (genomic instability) and/or trigger the transfer of signals from damaged cells to their undamaged neighbours (bystander effects); both genomic instability and bystander effects have been suggested as possible factors that modify radiation-associated cancer risk (UNSCEAR 2010). While the phenomenon of adaptive response cannot be denied, the explanation or mechanism is the subject of debate.

Objective of the study

To analyze the frequency of MN, CAs and DNA damage in blood samples of high-background natural radiation (HBNR) and normal-background radiation (NBR) sand-acclimatised rats using cytogenetic techniques.

Description of the study area

The southwest coast of Tamilnadu, India from north of Muttom to Colachel is considered to be a HBNR area due to the presence of monazite content in the soil which is rich in thorium, varying from 0.3% to 6%. The study was carried out in four villages during the period 2013–2014. Two coastal villages – Chinnavilai (08°08′216″N, 77°18′268″E) and Kadiapattanam (08°08′217″N, 77°18′270″E) were HBNR areas and other two – Manakudy (08°05′170″N, 77°29′146″E) and Rajakkamangalam Thurai (08°06′522″N, 77°22′366″E), as NBR areas (Fig. 1).

In this region, the HBNRA extends to a length of about 6 km, from the north of Muttom to Colachel, with the black sand bed having an average width of 45 m. One of the distinguishing features of the study villages is the type of settlement comprising clusters of houses. Each village has 1000-1500 houses. People of these villages are involved in traditional jobs like agriculture, farming and fishing, which expose them constantly to the monazite-rich soil. They have identical social, religious, economic and linguistic characteristics. Most of the able-bodied adult men are engaged in fishing. These villages have churches, schools and community centers. The major crop grown here is the coconut. Open wells are the major source of drinking water. Public water supply is also available in some areas. Fish is a major part of the diet. The radioactive elements such as uranium and thorium series present in soil and water, contribute to the high background radiation exposure to the population.

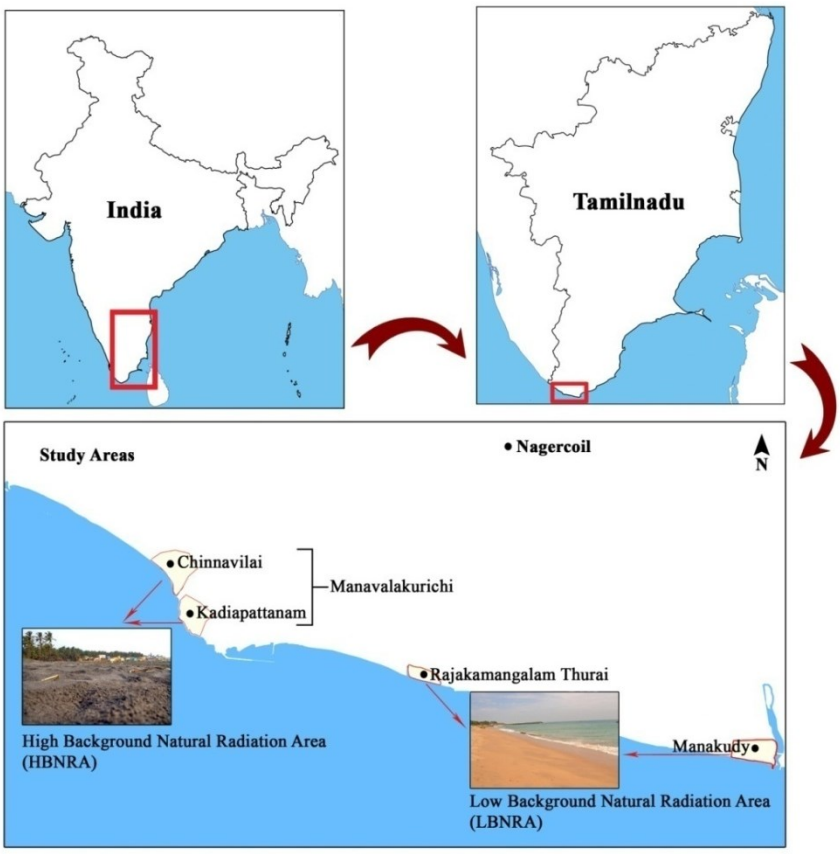


Fig. 1. Study area

The normal background radiation areas are Manakudy and Rajakkamangalam Thurai situated between the coastal regions of Pillaithoppu and Kanyakumari.

CHAPTER II
MATERIALS AND METHODS

2.1 Preparation of sand bed

Two locations with high and normal natural radioactivity (measured from 1 m above the ground level) were chosen. The HBNRA sand samples were collected from Kadiapattanam (8°08′140″N, 77°18′154″E) and NBRA sand samples from Rajakkamangalam Thurai (8°06′519″N, 77°22′374″E). The samples were scooped out from a depth of 5–30 cm by using a clean, hand shovel. About 100 kg of sand sample was collected from each location for the preparation of sand bed. Samples were collected in clean polythene bags for easy transportation to the laboratory. Two rectangular tanks of dimension 250 × 150 × 150 cm (L × B × H) were filled with sand, up to a height of 60 cm (monazite-rich black sand and normal sand). Above the sand bed a polythene sheet was spread to prevent the animals from direct contact with the sand. The tanks were kept in the animal house of the research center and the ambient temperature was maintained at 27±3°C.

2.2 Measurement of radiation level

The radiation levels of the sand beds were recorded by using the micro-R Survey meter (Nucleonix) measuring micro rad per hour (μR/h), with an accuracy of ±10, calibrated by Environmental Survey Laboratory, Kudankulam, Tamilnadu.

The measured radiation level was converted to absorbed radiation dose (mGy/year) using the following formula

R= Rad

1 μR = 0.01 μGy

1 μGy = 0.001 mGy

Absorbed radiation dose (μGy h^{-1}) = $\frac{\mu R/h}{100}$ = μGy/h

Absorbed radiation dose (mGy h^{-1}) = $\frac{\mu Gy/h}{1000}$ = mGy/h

Absorbed radiation dose (mGy y^{-1}) = mGy/h × 24 hours × 365.25 days × 1

1 = CF (Conversion factor) for air kerma for organ-specific absorbed dose [International Commission of Radiological Protection Report 74 (ICRP 1997)]

2.3 Animals

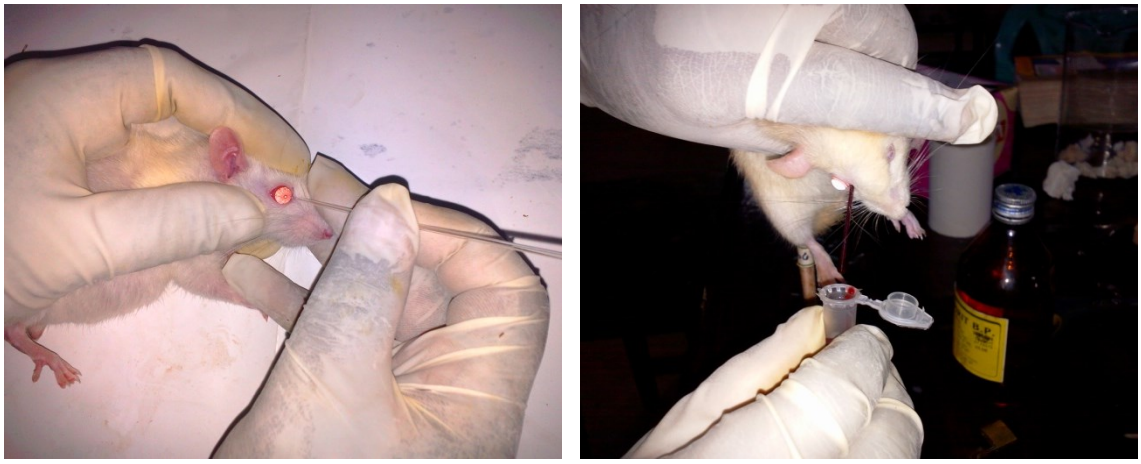
Wistar rats (outbred albino rat), aged between 2 and 3 weeks, weighing ~100 g, were purchased from a local vendor. The rats were maintained in well-ventilated acrylic cages with adequate water and rat feed until 1 month of age. The 1-month-old rats were tagged with coded metal ear tags for identification. The animals were then transferred to HBNR and NBR sand beds. A total of 45 young male Wistar rats (30 and 15 for HBNR and NBR sand beds, respectively) were studied. The rats were exposed to HBNR and NBR sand beds for a period of 24 months.

2.4 Survival and mortality rate of animals

The survival rate of the 45 Wistar rats was calculated at an interval of every 3 months, up to 24 months.

2.5 Blood collection and handling

Peripheral blood sample was collected from the retro-orbital sinus of the Wistar rats by using standard heparinized micro-hematocrit capillary tubes. The animal was held by the back of the neck and the loose skin of the head was tightened with the thumb and middle finger. The tip of the capillary tube was placed at the medial canthus of the eye under the nictitating membrane. A short thrust past the eyeball led to the entry of the capillary tube into the slightly resistant membrane of the sinus. The eyeball itself remained uninjured. As soon as the sinus was punctured, blood entered the tube by capillary action. The maximum amount of blood was collected in lithium heparin tube, EDTA tube and plain tube (tubes vary according to the tests), and the tube was withdrawn and slight pressure with a piece of gauze on the eyeball was exerted to prevent further bleeding (Fig. 2). The blood withdrawn each time from this location was 1% of the animal's body weight (e.g., 2.0 ml from a 200-g adult rat). Anesthesia was required for retro-orbital bleeding. For this, ketamine (75 mg/kg) was given as intraperitoneal (IP) injection. Prior to and during the procedure the respiratory rate, response to noxious stimulus, spontaneous movement and anesthesia-recovery-monitoring parameters were monitored at 5-min intervals. During recovery from anesthesia, respiratory rate, movement and ability to maintain sternal recumbancy were monitored at 5-min intervals until the animal was ambulatory. To protect the animals from hypothermia, they were placed on a water-recirculating covered well, to conserve body temperature. The animals recovered within 30-60 min postoperatively.



A B

Fig 2. (A) Retro-orbital sinus puncture, and (B) blood collection from rat using the standard heparinized micro-hematocrit capillary tubes

2.6 Blood collection interval

The blood samples were collected at different intervals based on the techniques applied. The details of blood collections are tabulated below (Table 1).

Table 1. Blood collection interval used for performing different techniques

Techniques	Blood collection time interval (months)	Blood withdrawn[a] (ml)	Collection tube
Cytokinesis-block micronucleus (CBMN) assay	3, 6, 9, 12, 15 and 18	0.5	5-ml Lithium Heparin tube
Chromosomal aberration analysis	6, 12 and 18	0.5	5-ml Lithium Heparin tube
Comet assay	9 and 18	0.5	5-ml EDTA tube

[a] Maximum quantity of blood withdrawn during single blood collection per animal

2.7 Composition of culture medium

Cells were cultured in the aqueous growth medium RPMI-1640 (Sigma-Aldrich) for chromosomal studies. The culture medium was balanced with salt solutions, glucose and buffering system to maintain proper pH, and supplemented

with L-glutamine, an amino acid essential for cell growth. Supplements such as fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin) were also added.

2.8 Preparation of culture medium

Culture medium was prepared under sterile conditions by using the laminar air flow chamber. Horizontal airflow chamber was used, as it is recommended for cell culture media preparation. For 100 ml of culture medium, 69 ml of RPMI-1640 supplemented with L-glutamine, 30 ml of FBS and 1 ml of antibiotics were taken in a sterile conical flask with screw cap. The culture medium was tightly packed to avoid contamination. The medium was used 3 days post-preparation to confirm the absence of contamination.

2.9 Cytokinesis-block micronucleus (CBMN) assay

Ionizing radiation induces the formation of acentric chromosome fragments and to a small extent malsegregation of whole chromosomes. Acentric chromosome fragments and whole chromosomes that are unable to interact with the spindle lag behind at anaphase, and as a result they are not included in the main daughter nuclei. A lagging chromosome fragment or whole chromosome forms into a small separate nucleus (micronucleus).

Using cytochalasin-B (Cyt-B), Fenech and Morley (1985) were able to demonstrate that cells that had completed one nuclear division could be accumulated and recognized as binucleated (BN) cells. MN could then be specifically and efficiently scored in these BN cells while excluding nondividing mononuclear cells that were unable to express MN *in vitro*. Consequently, the results obtained with the MN assay were not confounded by inter-individual and inter-experimental variation in

the frequency of dividing cells, which has been shown to have a profound effect on the observed MN frequency. The resulting CBMN assay has since become the standard method for measuring MN in cultured lymphocytes.

2.9.1 *Protocol*

About 0.5 ml of whole-blood sample was added to 4.5 ml of RPMI-1640 (Sigma-Aldrich) supplemented with L-glutamine, 30% heat-inactivated FBS and antibiotics (penicillin/streptomycin – 100 U/ml) in a sterile culture flask with screw cap. 100 µl of phytohaemagglutinin (PHA), at the concentration of 20 µg/ml, was added to the culture tube. The flasks were kept in a slanting position at 37°C in an incubator. 20 µl of Cyt-B was added to the culture, 24 h post-PHA stimulation, to give a final concentration of 6 µg/ml. This was the optimum concentration for accumulating BN cells in whole-blood cultures. The culture was terminated at 72 h post-PHA stimulation. Then the cells were gently centrifuged at 1000 rpm for 10 min and the supernatant was removed. The cells were hypotonically treated with 7 ml of cold (4°C) 0.075 M KCl and centrifuged immediately at 1000 rpm for 10 min. The supernatant was removed and replaced with 5 ml freshly made fixative consisting of methanol: acetic acid (3:1). The fixative was added whilst agitating the cells to prevent clump forming. The cells were washed with freshly prepared fixative two to three times and the supernatant removed 1 cm above the cell pellet; cells were resuspended gently, and the suspension dropped onto clean glass slides and allowed to air-dry. The slides were stained in 4% Giemsa's azur-eosin-methylene blue solution (Merck) in HEPES buffer (0.03 M; pH 6.5) for 15 min under dark conditions, followed by a quick rinse in distilled water and air-dried. The dried slides were used for enumeration under the light microscope.

2.9.2 *CBMN assay scoring criteria*

I. Criteria used for selecting binucleated cells

The cytokinesis-blocked cells that were scored for MN frequency had the following characteristics:

(a) The cells were binucleated (BN).

(b) The two nuclei in a BN cell had intact nuclear membranes and were situated within the same cytoplasmic boundary.

(c) The two nuclei in a BN cell were approximately equal in size, staining pattern and staining intensity.

(d) The two nuclei within a BN cell were unconnected or attached by one or more fine nucleoplasmic bridges, which were no wider than 1/4th of the nuclear diameter.

(e) The two main nuclei in a BN cell were touching but ideally not overlapping each other. A cell with two overlapping nuclei was scored only if the nuclear boundaries of either nucleus were distinguishable.

(f) The cytoplasmic boundary or membrane of a BN cell was intact and clearly distinguishable from the cytoplasmic boundaries of adjacent cells.

II. Criteria used for scoring micronuclei

MN were morphologically identical to but smaller than the main nuclei. They also had the following characteristics:

(a) The diameter of MN in human lymphocytes usually varied between $1/16^{th}$ and $1/3^{rd}$ of the mean diameter of the main nuclei, which corresponds to $1/256^{th}$ and $1/9^{th}$ of the area of one of the main nuclei in a BN cell, respectively.

(b) MN were non-refractile and can therefore be readily distinguished from artefacts such as staining particles.

(c) MN were not linked or connected to the main nuclei.

(d) MN were touching but not overlapping the main nuclei and the micronuclear boundary.

(e) MN had the same staining intensity as the main nuclei but occasionally staining was more intense.

2.10 Chromosomal aberration

A CA is a missing, extra, or irregular portion of chromosomal DNA. It can be from an atypical number of chromosomes or a structural abnormality in one or more chromosomes. The present study aimed at analyzing the structural abnormalities in chromosomes of rats exposed to background radiation.

2.10.1 *Protocol*

About 0.5 ml of whole-blood sample was added to 4.5 ml of RPMI-1640 supplemented with L-glutamine, 30% heat-inactivated FBS and antibiotics in a sterile culture flask with a screw cap. 100 µl of PHA at the concentration of 20 µg/ml was added to the culture tube. The flasks were kept in a slanting position at 37°C in an incubator. 50 µl of colchicine was added to the culture, 70 h post-PHA stimulation, to give a final concentration of 0.1 µg/ml. The culture was terminated 72 h post-PHA

stimulation. The harvest time was after 2 h of addition of colchicine to get cells at metaphase. Then the cells were gently centrifuged at 1000 rpm for 10 min and the supernatant culture medium was removed. The cells were hypotonically treated with 7 ml of cold (4°C) 0.075 M KCl and centrifuged immediately at 1000 rpm for 10 min. The supernatant was removed and replaced with 5 ml of freshly made fixative consisting of methanol: acetic acid (3:1). The fixative was added whilst agitating the cells to prevent clump formation. The cells were washed with freshly prepared fixative two to three times and the supernatant was removed 1 cm above the cell pellet; cells were resuspended gently, and the suspension dropped onto clean glass slides and allowed to air-dry. The slides were stained in 4% Giemsa's azur-eosin-methylene blue solution (Merck) in HEPES buffer (0.03 M; pH 6.5) for 15 min under dark conditions, followed by a quick rinse in distilled water and air-dried. The dried slide was used for CA studies.

2.10.2 *Karyotyping - G banding*

Giemsa bands (G bands) were obtained by digesting the chromosome with the proteolytic enzyme trypsin. The slides were 'aged' overnight at 60°C in a hot air oven to enhance chromosome banding. The slides were treated with trypsin solution (0.01%) for 5 to 10 s, followed by rinsing with Sorenson's buffer. The treated slides were stained with 10% Giemsa staining solution for 2 min (5 ml Giemsa + 45 ml Sorensen's buffer). The slides were rinsed in distilled water and allowed to dry. The slides were mounted using DPX and examined under oil-immersion objectives.

2.11 Comet assay

Comet assay was done by using OxiSelect™ 96-Well Comet Assay Kit manufactured by Cell Biolabs, Inc. This kit is a single-cell gel electrophoresis assay (SCGE) for evaluation of cellular DNA damage. The individual cells were mixed with molten agarose before application to the OxiSelect™ 96-Well Comet Slide. These embedded cells were then treated with a lysis buffer and alkaline solution, which relaxes and denatures the DNA. Finally, the samples were electrophoresed in a horizontal chamber to separate intact DNA from damaged fragments. Following electrophoresis, the samples were dried, stained with a DNA dye, and visualized by epifluorescence microscopy. Under these conditions, the damaged DNA (containing cleavage and strand breaks) will migrate further than intact DNA and produce a 'comet tail' shape.

2.11.1 *Preparation of Reagents*

i. **OxiSelect™ Comet Agarose:** The bottle containing the comet agarose was heated at 90-95°C in a water bath for 20 min. Then the bottle was transferred to a 37°C water bath for 20 min and the temperature maintained until slide preparation.

ii. **Vista Green DNA Dye:** A 1X Vista Green DNA staining solution was prepared by diluting the stock 1:10000 in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA). The solution was stored at 4°C, and protected from light.

iii. **Lysis Buffer:** 100 ml of 1X lysis buffer was prepared by using the following reagents

NaCl	14.6 g
EDTA Solution	20.0 ml
10X Lysis Solution	10.0 ml
DMSO	10.0 ml
Distilled water	Adjust volume to 90 ml

NaCl was mixed thoroughly in distilled water. The lysis buffer was adjusted to pH 10.0 with 10 N NaOH; then the solution was made up to 100 ml by using distilled water. The lysis buffer was chilled to 4°C before use.

iv. **Alkaline Solution:** 100 ml of alkaline solution was prepared by using the following reagents

NaOH	1.2 g
EDTA Solution	0.2 ml
Distilled water	Adjust volume to 100 ml

Alkaline solution was chilled to 4°C before use.

v. Alkaline Electrophoresis Solution (300 mM NaOH, pH >13, 1 mM EDTA)

Preparation of 1 L of electrophoresis solution

NaOH	12.0 g
EDTA Solution	2.0 ml
Distilled water	Adjust volume to 1 L

Alkaline running solution was chilled to 4°C before use.

2.11.2 *Preparation of samples*

Peripheral blood mononuclear cells (PBMCs) were isolated from the whole-blood samples by using Histopaque 1077 (Sigma–Aldrich).

i. To a 15-ml conical centrifuge tube, 3 ml of Histopaque-1077 was added and brought to room temperature.

ii. A volume of 3 ml of whole blood was carefully layered onto the Histopaque-1077.

iii. This mixture was centrifuged at 2000 rpm for exactly 30 min at room temperature.

iv. After centrifugation, the upper layer was carefully aspirated with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells. The upper layer was then discarded.

v. The opaque interface was carefully transferred by using a Pasteur pipette into a clean conical centrifuge tube.

vi. The cells were washed by adding 10 ml of isotonic phosphate-buffered saline solution, and mixed by gently drawing in and out of a Pasteur pipette.

vii. The mixture was centrifuged at 1500 rpm for 10 min.

viii. The supernatant was aspirated and discarded.

ix. The cell pellet was resuspended with 5 ml of isotonic phosphate-buffered saline solution, and mixed by gently drawing in and out of a Pasteur pipette.

x. The mixture was again centrifuged at 1500 rpm for 10 min.

xi. Steps viii, ix and x were repeated, the supernatant discarded and cell pellet resuspended in 0.5 ml of isotonic phosphate-buffered saline solution.

2.11.3 *Preparation of slides*

i. A volume of 20 µl of pre-heated comet agarose was added per well onto the OxiSelect™ 96-Well Comet Slide to create a base layer. Complete well coverage was ensured by spreading the solution over the well with the pipette tip. The slide was chilled at 4°C for 15 min.

ii. Isolated cell samples were added to pre-heated comet agarose at 1:10 ratio (v/v). This mixture was mixed well by pipetting, and 20 µl was immediately transferred per well onto the top of the comet agarose base layer by using a micropipette.

iii. The slide was maintained horizontally, and chilled at 4°C under dark conditions for 15 min.

iv The slide was transferred to a container containing pre-chilled lysis buffer (~50-100 ml/slide) and the slide immersed in the buffer for 30-60 min at 4°C under dark conditions.

v. The lysis buffer was aspirated from the container and replaced with pre-chilled alkaline solution (~50-100 ml/slide). The slide was immersed in the solution for 30 min and chilled at 4°C under dark conditions.

2.11.4 ***Assay Protocol - Alkaline Electrophoresis***

i. The slide was maintained horizontally, transferred from the alkaline solution to a horizontal electrophoresis chamber. The chamber was filled with cold alkaline electrophoresis solution until the buffer level covered the slide.

ii. Voltage was applied to the chamber for 15-30 min at 1 V/cm. Additionally, the volume of the alkaline electrophoresis solution was adjusted to produce a current of 300 mA.

iii. The slide was maintainined horizontally, and transferred from the electrophoresis chamber to a clean container containing pre-chilled distilled water (~50-100 ml/slide). The slide was immersed for 2 min, aspirated, and the procedure repeated twice.

iv. The final water rinse was aspirated and replaced with cold 70% ethanol for 5 min.

v. The slide was maintained horizontally, removed from 70% ethanol and allowed to air-dry.

vi. Once the agarose and slide were completely dried, 50 µl/well of diluted Vista Green DNA Dye was added and the slide incubated at room temperature for 15 min.

vii. The slides were viewed by epifluorescence microscopy by using an FITC filter.

2.11.5 *Analysis and calculation*

The images obtained using epifluorescence microscopy were analyzed using Image J (Open comet) v1.50i software (Fig. 3). The software auto calculated comet length, head length, tail length, tail DNA intensity, tail DNA% and tail moment.

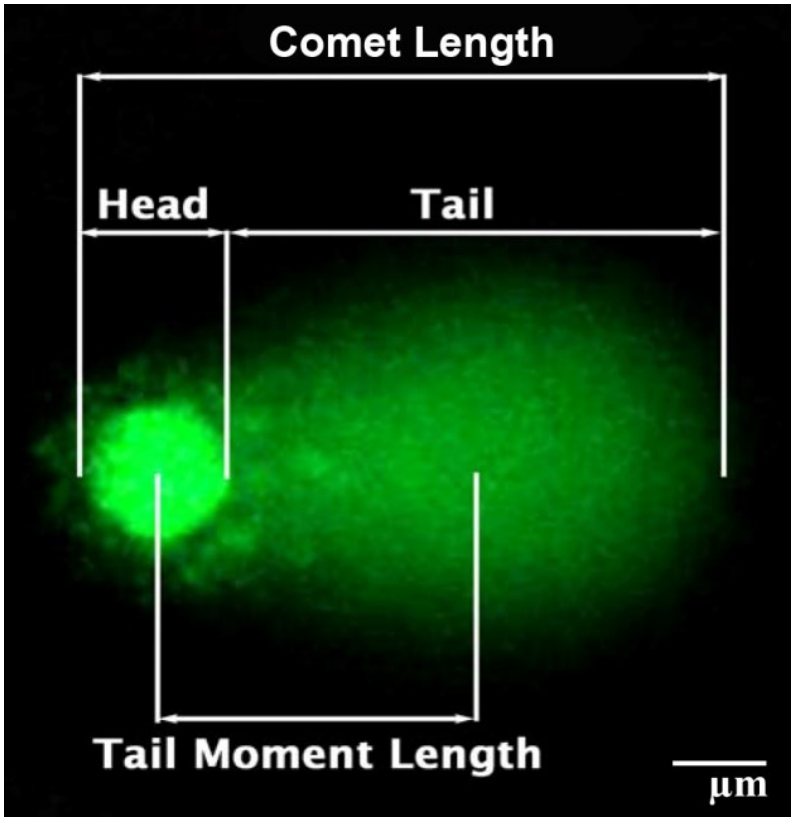


Fig. 3. Measurement of comet in DNA damage analysis

2.12 Statistical analysis

Statistical analyses were carried out by using the statistical software SPSS v11. ANOVA was used to compare the frequency of MN, CAs and tail moment

(comet asssay) in HBNR- and NBR-sand-exposed groups. The frequency of MN and CAs at different age groups in HBNR- and NBR-exposed rats was also analyzed by using ANOVA. The Bonferroni *post hoc* test was applied for the multiple comparison of age groups. Regression analysis was also done for age-dependent analysis.

CHAPTER III
RESULTS AND DISCUSSION

3.1 Measurement of radiation level in prepared sand bed

The radiation level of sand beds was recorded at the height of 0.5 m using the micro-R Survey meter (Nucleonix) (microgray per hour; μGy/h) and results are given in Table 2. The measured radiation dose values were converted to milligray (mGy). The radiation dose for every 3 months up to 24 months was calculated (Table 3).

Table 2. Measurement of radiation level in HBNR and NBR sand bed

Sand bed	**Radiation level** (μGy/h)					**Mean**	**SD**
	Readings						
	1	**2**	**3**	**4**	**5**		
HBNR	10.76	9.97	10.32	11.03	10.92	**10.60**	**0.44**
NBR	0.27	0.31	0.29	0.23	0.26	**0.27**	**0.03**

Table 3. Measurement of radiation dose absorbed by rats in different timelines

Sand bed	**Radiation dose** (mGy/year)		**Radiation dose absorbed by rats in different timelines** (mGy; months)						
	Mean	**SD**	**3**	**6**	**9**	**12**	**15**	**18**	**24**
HBNR	92.91	3.89	23.23 ± 0.97	46.46 ± 1.95	69.69 ± 2.92	92.91 ± 3.89	116.15 ± 4.86	139.38 ± 5.84	185.82 ±7.78
NBR	2.38	0.26	0.60 ± 0.07	1.19 ± 0.13	1.79 ± 0.20	2.38 ± 0.26	2.98 ± 0.33	3.58 ± 0.40	4.76 ± 0.52

3.2 Survival and mortality rate

The rats exposed to HBNR and NBR sand beds were counted to estimate survival and mortality rate. The survival and mortality rate of animals was calculated by using the below-mentioned formulae. The results are presented in Table 4.

$$\text{Survival rate (\%)} = \frac{\text{Total number of rats survived}}{\text{Total number of rats exposed}} \text{x}100$$

$$\text{Mortality rate (\%)} = \frac{\text{Total number of rats died}}{\text{Total number of rats exposed}} \text{x}100$$

Table 4. Survival and mortality rate of rats exposed to HBNR and NBR sand bed at 3-month intervals.

Exposure time frame (months)	Anticipated number of animals to be exposed		Actual number of animals exposed		Survival rate (%)	Mortality rate (%)
	HBNR sand bed	NBR sand bed	HBNR sand bed	NBR sand bed		
3	30	15	30	15	100	0
6	30	15	30	15	100	0
9	30	15	29	15	97.8	2.2
12	30	15	27	14	91.1	8.9
15	30	15	26	14	88.9	11.1
18	30	15	24	12	80.0	20.0
21	30	15	23	12	77.8	22.2
24	30	15	21	11	71.1	28.9

3.3 Cytokinesis-block micronucleus (CBMN) assay

The CBMN assay was carried out in Wistar rats. Thirty rats exposed to HBNR sand and 15 rats exposed to NBR sand (control) were examined. Blood samples drawn at an interval of 3 months up to 18 months were analyzed for MN frequency. The distribution and frequency of MN in peripheral blood lymphocytes were noted (Tables 5 to 16). The distribution of MN was counted based on number of MN present in a single bi-nucleated cell (Fig. 4).

In HBNR sand-exposed rats the frequency of cells with MN was observed to be 15.0±2.9, 16.7±4.4, 17.2±4.6, 17.1±4.9, 18.5±5.1 and 20.3±4.4 per 1000 BN cells, for the dose rate of 23.23±0.97 mGy (3 months), 46.46±1.95 mGy (6 months), 69.69±2.92 mGy (9 months), 92.91±3.89 mGy (12 months), 116.15±4.86 mGy (15 months) and 139.38±5.84 mGy (18 months), respectively, whereas it was 15.3±3.0, 16.4±3.9, 16.9±3.7, 17.5±3.8, 17.9±3.7 and 19.7±3.8 per 1000 BN cells from NBR sand exposed rats at the dose rate of 0.60±0.07 mGy (3 months), 1.19±0.13 mGy (6 months), 1.79±0.20 mGy (9 months), 2.38±0.26 mGy (12 months), 2.98±0.33 mGy (15 months) and 3.58±0.40 mGy (18 months) respectively (Table 17, Fig. 5). ANOVA did not show any statistical difference in MN frequency between HBNR and NBR exposed rats for 3 months [$F(1,43) = 0.062$, $P > 0.05$], 6 months [$F(1,43) = 0.062$, $P > 0.05$], 9 months [$F(1,42) = 0.075$, $P > 0.05$], 12 months [$F(1,39) = 0.067$, $P > 0.05$], 15 months [$F(1,38) = 0.156$, $P > 0.05$], and 18 months [$F(1,34) = 0.173$, $P > 0.05$]. On the one hand, this may be due to the fact that the level of radiation exposure was too low to induce significant MN. On the other hand, it is possible that damage had been induced, but repaired, in rats exposed to high background radiation sand.

However, an age-dependent increase in the frequency of MN was observed (Table 18). Analysis of variance suggested that the difference in frequencies were statistically significant among age groups [$F(5,160) = 4.313$, $P < 0.05$]. *Post-hoc* analysis of MN frequencies in the age groups revealed that the mean difference is significant at the 0.05 level. Linear regression analysis of the data also showed a significant increase in the frequency of MN with respect to age [$Y = 0.2831X + 14.157$; $R^2 = 0.1089$, $P < 0.05$ (Fig. 6)]. The age-related increase in the frequency of MN in rats was similar in HBNR and NBR sand-exposed rats [$F(5,79) = 2.218$, $P < 0.05$; Table 19)].

Similar to the results of this study, Karuppasamy *et al*. (2016), Thampi *et al*. (2005) and Cheriyan *et al*. (1999) reported statistically comparable frequencies of MN among individuals in high background radiation areas of Kerala. Mohammadi *et al*. (2006) reported no significant difference in basal MN frequencies between individuals from high background radiation areas of Ramsar, Iran and control areas. However an age-dependent increase in MN was seen in the present study. Age-dependent increase was most likely due to an increase in acentric fragments produced by unrepaired DNA strand-breaks induced either by exposure to environmental pollutants or endogenously or by spindle disturbances resulting in chromosome lagging (Fenech *et al*., 2011). It has been reported that, in old age, biomarkers of genomic instability, such as MN, are more common in the peripheral lymphocytes (Kažimírová *et al*., 2009). An age-dependent increase in the spontaneous MN frequency in bone marrow of Swiss mice was observed indicating DNA damage in peripheral blood, which was also reflected in tail-moment values obtained with the comet assay (Bhilwade *et al*., 2014). Accumulation of DNA damage at older age could be due to reduced repair capacity with age and a high receptiveness to DNA damage upon external environmental exposure (Bohr *et al*., 1995; Walter *et al*., 1997). Gender-related differences in MN frequency could not be evaluated, since only male rats were analyzed for MN frequency.

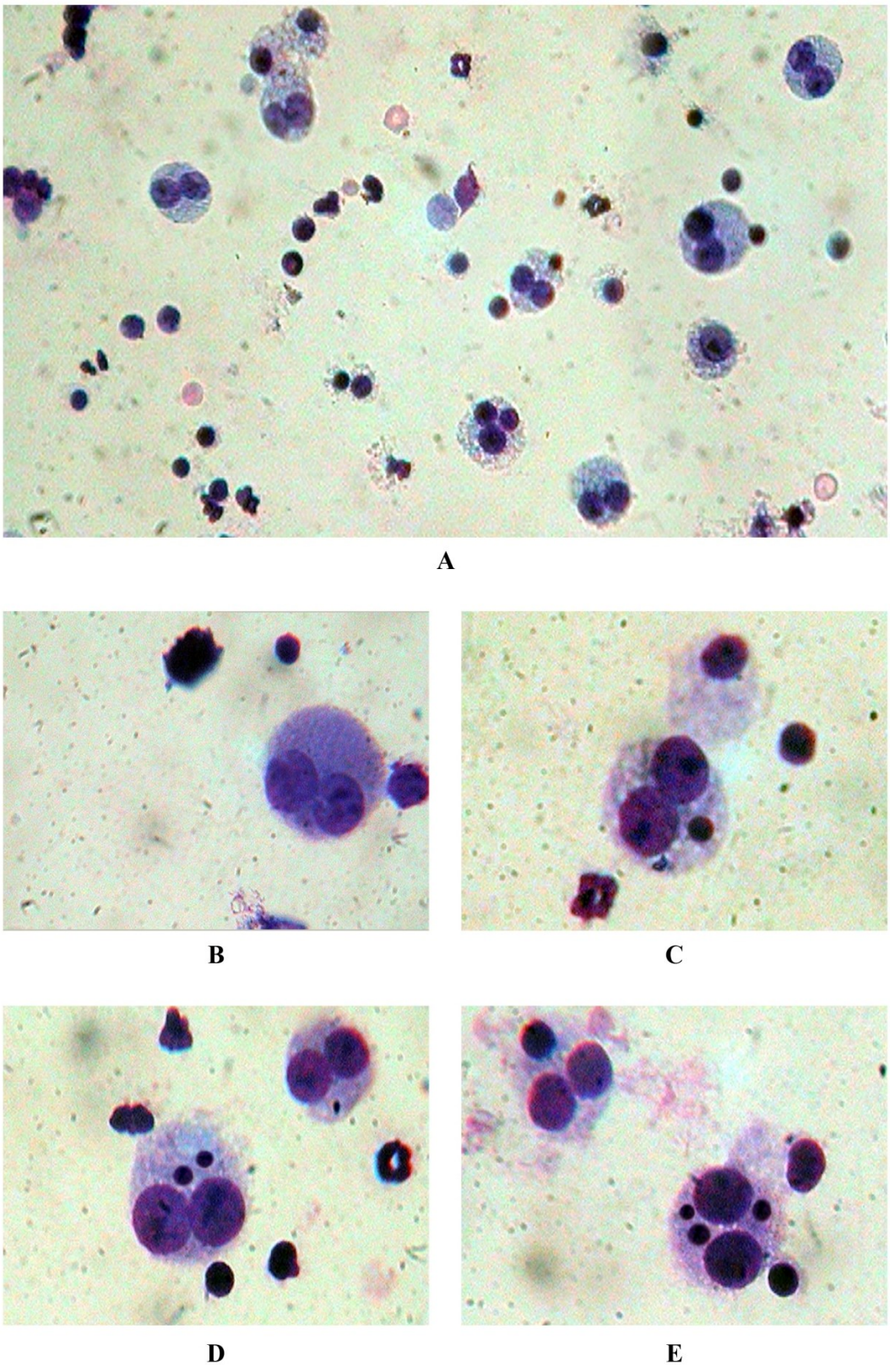


Fig 4. Microscopic field showing (A) group of binucleated cells in a single field, (B) BN cell without MN, (C) BN cell 1MN, (D) BN cell 2MN, (E) BN cell 3MN [Magnification - image (A) - ×100 and image (B),(C),(D),(E) - ×400]

Table 5. Distribution and frequencies of MN in peripheral lymphocytes of 3-month HBNR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 3 months radiation exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
H1	23.23 ± 0.97	11	989	11			0.011
H2	23.23 ± 0.97	17	985	13	2		0.017
H3	23.23 ± 0.97	15	985	15			0.015
H4	23.23 ± 0.97	18	983	16	1		0.018
H5	23.23 ± 0.97	16	985	14	1		0.016
H6	23.23 ± 0.97	12	988	12			0.012
H7	23.23 ± 0.97	18	982	18			0.018
H8	23.23 ± 0.97	15	986	13	1		0.015
H9	23.23 ± 0.97	15	985	15			0.015
H10	23.23 ± 0.97	9	991	9			0.009
H11	23.23 ± 0.97	17	985	13	2		0.017
H12	23.23 ± 0.97	14	986	14			0.014
H13	23.23 ± 0.97	20	981	18	1		0.020
H14	23.23 ± 0.97	20	980	20			0.020
H15	23.23 ± 0.97	10	990	10			0.010
H16	23.23 ± 0.97	14	986	14			0.014
H17	23.23 ± 0.97	18	985	13	1	1	0.018
H18	23.23 ± 0.97	14	986	14			0.014
H19	23.23 ± 0.97	15	985	15			0.015
H20	23.23 ± 0.97	15	987	11	2		0.015
H21	23.23 ± 0.97	19	982	17	1		0.019
H22	23.23 ± 0.97	15	985	15			0.015
H23	23.23 ± 0.97	14	986	14			0.014
H24	23.23 ± 0.97	16	984	16			0.016
H25	23.23 ± 0.97	17	986	11	3		0.017
H26	23.23 ± 0.97	16	984	16			0.016
H27	23.23 ± 0.97	14	987	12	1		0.014
H28	23.23 ± 0.97	9	991	9			0.009
H29	23.23 ± 0.97	15	985	15			0.015
H30	23.23 ± 0.97	13	987	13			0.013
N=30	**Mean**	**15.0**				**Mean**	**0.015**
	SD	**2.9**				**SD**	**0.003**

Table 6. Distribution and frequencies of MN in peripheral lymphocytes of 3-month NBR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 3 months exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
N1	0.60 ± 0.07	16	985	14	1		0.016
N2	0.60 ± 0.07	21	980	19	1		0.021
N3	0.60 ± 0.07	10	990	10			0.010
N4	0.60 ± 0.07	16	984	16			0.016
N5	0.60 ± 0.07	17	984	15	1		0.017
N6	0.60 ± 0.07	17	983	17			0.017
N7	0.60 ± 0.07	17	985	13	2		0.017
N8	0.60 ± 0.07	13	988	11	1		0.013
N9	0.60 ± 0.07	14	986	14			0.014
N10	0.60 ± 0.07	15	987	11	2		0.015
N11	0.60 ± 0.07	9	991	9			0.009
N12	0.60 ± 0.07	15	985	15			0.015
N13	0.60 ± 0.07	18	982	18			0.018
N14	0.60 ± 0.07	17	984	15	1		0.017
N15	0.60 ± 0.07	14	986	14			0.014
N=15	**Mean**	**15.3**				**Mean**	**0.015**
	SD	**3.0**				**SD**	**0.003**

Table 7. Distribution and frequencies of MN in peripheral lymphocytes of 6-month HBNR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 6 months radiation exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
H1	46.46 ± 1.95	22	979	20	1		0.022
H2	46.46 ± 1.95	14	986	14			0.014
H3	46.46 ± 1.95	14	987	12	1		0.014
H4	46.46 ± 1.95	19	984	14	1	1	0.019
H5	46.46 ± 1.95	17	984	15	1		0.017
H6	46.46 ± 1.95	11	989	11			0.011
H7	46.46 ± 1.95	16	986	12	2		0.016
H8	46.46 ± 1.95	9	991	9			0.009
H9	46.46 ± 1.95	28	977	19	3	1	0.028
H10	46.46 ± 1.95	17	983	17			0.017
H11	46.46 ± 1.95	16	984	16			0.016
H12	46.46 ± 1.95	19	983	15	2		0.019
H13	46.46 ± 1.95	19	982	17	1		0.019
H14	46.46 ± 1.95	18	982	18			0.018
H15	46.46 ± 1.95	10	990	10			0.010
H16	46.46 ± 1.95	20	982	16	2		0.020
H17	46.46 ± 1.95	18	984	14	2		0.018
H18	46.46 ± 1.95	18	983	16	1		0.018
H19	46.46 ± 1.95	14	986	14			0.014
H20	46.46 ± 1.95	19	984	13	3		0.019
H21	46.46 ± 1.95	23	978	21	1		0.023
H22	46.46 ± 1.95	17	983	17			0.017
H23	46.46 ± 1.95	15	986	13	1		0.015
H24	46.46 ± 1.95	12	988	12			0.012
H25	46.46 ± 1.95	18	983	16	1		0.018
H26	46.46 ± 1.95	19	982	17	1		0.019
H27	46.46 ± 1.95	23	978	21	1		0.023
H28	46.46 ± 1.95	9	991	9			0.009
H29	46.46 ± 1.95	17	985	13	2		0.017
H30	46.46 ± 1.95	11	989	11			0.011
N=30	**Mean**	**16.7**				**Mean**	**0.017**
	SD	**4.4**				**SD**	**0.004**

Table 8. Distribution and frequencies of MN in peripheral lymphocytes of 6-month NBR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 6 months exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
N1	1.19 ± 0.13	16	984	16			0.016
N2	1.19 ± 0.13	15	986	13	1		0.015
N3	1.19 ± 0.13	20	981	18	1		0.020
N4	1.19 ± 0.13	12	988	12			0.012
N5	1.19 ± 0.13	12	989	10	1		0.012
N6	1.19 ± 0.13	13	987	13			0.013
N7	1.19 ± 0.13	20	982	16	2		0.020
N8	1.19 ± 0.13	17	984	15	1		0.017
N9	1.19 ± 0.13	13	987	13			0.013
N10	1.19 ± 0.13	15	985	15			0.015
N11	1.19 ± 0.13	14	986	14			0.014
N12	1.19 ± 0.13	20	980	20			0.020
N13	1.19 ± 0.13	26	978	19	2	1	0.026
N14	1.19 ± 0.13	14	986	14			0.014
N15	1.19 ± 0.13	19	983	15	2		0.019
N=15	**Mean**	**16.4**				**Mean**	**0.016**
	SD	**3.9**				**SD**	**0.004**

Table 9. Distribution and frequencies of MN in peripheral lymphocytes of 9-month HBNR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 9 months radiation exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
H1	69.69 ± 2.92	19	981	19			0.019
H2	69.69 ± 2.92	19	982	17	1		0.019
H3	69.69 ± 2.92	14	987	12	1		0.014
H4	69.69 ± 2.92	21	981	17	2		0.021
H5	69.69 ± 2.92	21	982	15	3		0.021
H6	69.69 ± 2.92	8	992	8			0.008
H7	69.69 ± 2.92	21	980	19	1		0.021
H8	69.69 ± 2.92	17	984	15	1		0.017
H9	69.69 ± 2.92	12	988	12			0.012
H10	69.69 ± 2.92	22	980	18	2		0.022
H11	69.69 ± 2.92	14	986	14			0.014
H12	69.69 ± 2.92	13	988	11	1		0.013
H13	69.69 ± 2.92	18	985	13	1	1	0.018
H14	69.69 ± 2.92	10	990	10			0.010
H15	69.69 ± 2.92	20	982	16	2		0.020
H16	69.69 ± 2.92	18	984	14	2		0.018
H17	69.69 ± 2.92	15	986	13	1		0.015
H18	69.69 ± 2.92	17	983	17			0.017
H19	69.69 ± 2.92	21	982	15	3		0.021
H20	69.69 ± 2.92	22	979	20	1		0.022
H21*							
H22	69.69 ± 2.92	27	977	19	4		0.027
H23	69.69 ± 2.92	10	990	10			0.010
H24	69.69 ± 2.92	20	982	16	2		0.020
H25	69.69 ± 2.92	13	987	13			0.013
H26	69.69 ± 2.92	20	983	14	3		0.020
H27	69.69 ± 2.92	23	981	16	2	1	0.023
H28	69.69 ± 2.92	11	990	9	1		0.011
H29	69.69 ± 2.92	18	985	12	3		0.018
H30	69.69 ± 2.92	16	985	14	1		0.016
N=29	**Mean**	**17.2**				**Mean**	**0.017**
	SD	**4.6**				**SD**	**0.004**
*Rat died during experimentation due to unknown reason; not able to obtain data.							

Table 10. Distribution and frequencies of MN in peripheral lymphocytes of 9-month NBR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 9 months exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
N1	1.79 ± 0.20	17	984	15	1		0.017
N2	1.79 ± 0.20	14	987	12	1		0.014
N3	1.79 ± 0.20	23	980	17	3		0.023
N4	1.79 ± 0.20	19	983	16		1	0.019
N5	1.79 ± 0.20	12	989	10	1		0.012
N6	1.79 ± 0.20	15	985	15			0.015
N7	1.79 ± 0.20	20	982	16	2		0.020
N8	1.79 ± 0.20	17	984	15	1		0.017
N9	1.79 ± 0.20	19	982	17	1		0.019
N10	1.79 ± 0.20	15	985	15			0.015
N11	1.79 ± 0.20	11	989	11			0.011
N12	1.79 ± 0.20	20	982	16	2		0.020
N13	1.79 ± 0.20	23	979	19	2		0.023
N14	1.79 ± 0.20	15	986	13	1		0.015
N15	1.79 ± 0.20	13	988	11	1		0.013
N=15	**Mean**	**16.9**				**Mean**	**0.017**
	SD	**3.7**				**SD**	**0.004**

Table 11. Distribution and frequencies of MN in peripheral lymphocytes of 12-month HBNR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 12 months radiation exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
H1	92.91 ± 3.89	18	983	16	1		0.018
H2	92.91 ± 3.89	13	987	13			0.013
H3	92.91 ± 3.89	16	985	14	1		0.016
H4	92.91 ± 3.89	21	982	15	3		0.021
H5	92.91 ± 3.89	15	987	11	2		0.015
H6	92.91 ± 3.89	16	985	14	1		0.016
H7	92.91 ± 3.89	11	989	11			0.011
H8	92.91 ± 3.89	15	986	13	1		0.015
H9*							
H10	92.91 ± 3.89	21	981	17	2		0.021
H11	92.91 ± 3.89	9	991	9			0.009
H12	92.91 ± 3.89	22	979	20	1		0.022
H13*							
H14	92.91 ± 3.89	16	985	14	1		0.016
H15	92.91 ± 3.89	12	989	10	1		0.012
H16	92.91 ± 3.89	25	977	21	2		0.025
H17	92.91 ± 3.89	19	982	17	1		0.019
H18	92.91 ± 3.89	15	985	15			0.015
H19	92.91 ± 3.89	29	976	20	3	1	0.029
H20	92.91 ± 3.89	14	987	12	1		0.014
H21*							
H22	92.91 ± 3.89	27	979	16	4	1	0.027
H23	92.91 ± 3.89	15	985	15			0.015
H24	92.91 ± 3.89	18	984	14	2		0.018
H25	92.91 ± 3.89	11	989	11			0.011
H26	92.91 ± 3.89	20	982	16	2		0.020
H27	92.91 ± 3.89	18	984	14	2		0.018
H28	92.91 ± 3.89	12	989	10	1		0.012
H29	92.91 ± 3.89	20	983	14	3		0.020
H30	92.91 ± 3.89	14	987	12	1		0.014
N=27	**Mean**	**17.1**				**Mean**	**0.017**
	SD	**4.9**				**SD**	**0.005**
*Rats died during experiment due to unknown reason, not able to obtain data.							

Table 12. Distribution and frequencies of MN in peripheral lymphocytes of 12-month NBR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 12 months exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
N1	2.38 ± 0.26	15	986	13	1		0.015
N2	2.38 ± 0.26	11	989	11			0.011
N3	2.38 ± 0.26	13	987	13			0.013
N4*							
N5	2.38 ± 0.26	16	985	14	1		0.016
N6	2.38 ± 0.26	19	981	19			0.019
N7	2.38 ± 0.26	24	978	20	2		0.024
N8	2.38 ± 0.26	17	984	15	1		0.017
N9	2.38 ± 0.26	19	982	17	1		0.019
N10	2.38 ± 0.26	19	984	13	3		0.019
N11	2.38 ± 0.26	14	986	14			0.014
N12	2.38 ± 0.26	18	982	18			0.018
N13	2.38 ± 0.26	16	985	14	1		0.016
N14	2.38 ± 0.26	20	980	20			0.020
N15	2.38 ± 0.26	24	980	17	2	1	0.024
N=14	**Mean**	**17.5**				**Mean**	**0.018**
	SD	**3.8**				**SD**	**0.004**
*Rat died during experiment due to unknown reason; not able to obtain data.							

Table 13. Distribution and frequencies of MN in peripheral lymphocytes of 15-month HBNR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 15 months radiation exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
H1	116.15 ± 4.86	26	978	19	2	1	0.026
H2	116.15 ± 4.86	19	982	17	1		0.019
H3	116.15 ± 4.86	12	988	12			0.012
H4	116.15 ± 4.86	19	984	13	3		0.019
H5	116.15 ± 4.86	16	986	12	2		0.016
H6	116.15 ± 4.86	13	988	11	1		0.013
H7	116.15 ± 4.86	9	991	9			0.009
H8	116.15 ± 4.86	19	982	17	1		0.019
H9*							
H10	116.15 ± 4.86	19	983	15	2		0.019
H11	116.15 ± 4.86	11	989	11			0.011
H12	116.15 ± 4.86	24	979	19	1	1	0.024
H13*							
H14*							
H15	116.15 ± 4.86	20	981	18	1		0.020
H16	116.15 ± 4.86	23	979	19	2		0.023
H17	116.15 ± 4.86	26	975	24	1		0.026
H18	116.15 ± 4.86	19	981	19			0.019
H19	116.15 ± 4.86	30	975	21	3	1	0.030
H20	116.15 ± 4.86	22	979	20	1		0.022
H21*							
H22	116.15 ± 4.86	23	981	15	4		0.023
H23	116.15 ± 4.86	14	986	14			0.014
H24	116.15 ± 4.86	17	984	15	1		0.017
H25	116.15 ± 4.86	20	983	14	3		0.020
H26	116.15 ± 4.86	17	985	13	2		0.017
H27	116.15 ± 4.86	21	980	19	1		0.021
H28	116.15 ± 4.86	16	984	16			0.016
H29	116.15 ± 4.86	13	988	11	1		0.013
H30	116.15 ± 4.86	14	987	12	1		0.014
N=26	**Mean**	**18.5**				**Mean**	**0.019**
	SD	**5.1**				**SD**	**0.005**
*Rats died during experiment due to unknown reason; not able to obtain data.							

Table 14. Distribution and frequencies of MN in peripheral lymphocytes of 15-month NBR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 15 months exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
N1	2.98 ± 0.33	19	982	17	1		0.019
N2	2.98 ± 0.33	23	980	17	3		0.023
N3	2.98 ± 0.33	11	989	11			0.011
N4*							
N5	2.98 ± 0.33	16	985	14	1		0.016
N6	2.98 ± 0.33	21	980	19	1		0.021
N7	2.98 ± 0.33	18	983	16	1		0.018
N8	2.98 ± 0.33	16	985	14	1		0.016
N9	2.98 ± 0.33	17	984	15	1		0.017
N10	2.98 ± 0.33	23	979	19	2		0.023
N11	2.98 ± 0.33	12	989	10	1		0.012
N12	2.98 ± 0.33	21	979	21			0.021
N13	2.98 ± 0.33	16	985	14	1		0.016
N14	2.98 ± 0.33	21	981	17	2		0.021
N15	2.98 ± 0.33	17	985	13	2		0.017
N=14	**Mean**	**17.9**				**Mean**	**0.018**
	SD	**3.7**				**SD**	**0.004**
*Rat died during experiment due to unknown reason; not able to obtain data.							

Table 15. Distribution and frequencies of MN in peripheral lymphocytes of 18-month HBNR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 18 months radiation exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
H1*							
H2	139.38 ± 5.84	29	977	19	2	2	0.029
H3*							
H4	139.38 ± 5.84	20	982	16	2		0.020
H5	139.38 ± 5.84	28	975	22	3		0.028
H6	139.38 ± 5.84	23	978	21	1		0.023
H7	139.38 ± 5.84	18	983	16	1		0.018
H8	139.38 ± 5.84	15	985	15			0.015
H9*							
H10	139.38 ± 5.84	23	981	16	2	1	0.023
H11	139.38 ± 5.84	19	981	19			0.019
H12	139.38 ± 5.84	16	985	14	1		0.016
H13*							
H14*							
H15	139.38 ± 5.84	24	977	22	1		0.024
H16	139.38 ± 5.84	21	982	15	3		0.021
H17	139.38 ± 5.84	22	979	20	1		0.022
H18	139.38 ± 5.84	20	983	14	3		0.020
H19	139.38 ± 5.84	14	986	14			0.014
H20	139.38 ± 5.84	15	986	13	1		0.015
H21*							
H22	139.38 ± 5.84	18	984	14	2		0.018
H23	139.38 ± 5.84	24	980	16	4		0.024
H24	139.38 ± 5.84	17	984	15	1		0.017
H25	139.38 ± 5.84	23	979	19	2		0.023
H26	139.38 ± 5.84	23	982	14	3	1	0.023
H27	139.38 ± 5.84	17	984	15	1		0.017
H28	139.38 ± 5.84	25	976	23	1		0.025
H29	139.38 ± 5.84	11	989	11			0.011
H30	139.38 ± 5.84	22	979	20	1		0.022
N=24	**Mean**	**20.3**				**Mean**	**0.020**
	SD	**4.4**				**SD**	**0.004**
*Rat died during experiment due to unknown reason; not able to obtain data.							

Table 16. Distribution and frequencies of MN in peripheral lymphocytes of 18-month NBR sand-exposed rats

Sample code	Radiation Dose (mGy)	Frequency of MN/1000 cells at 18 months exposure					
		No. MN	Distribution of MN				MN/cell
			0	1	2	3	
N1	3.58 ± 0.40	17	984	15	1		0.017
N2	3.58 ± 0.40	23	979	19	2		0.023
N3	3.58 ± 0.40	20	980	20			0.020
N4*							
N5	3.58 ± 0.40	13	988	11	1		0.013
N6	3.58 ± 0.40	26	977	21	1	1	0.026
N7	3.58 ± 0.40	22	980	18	2		0.022
N8	3.58 ± 0.40	18	982	18			0.018
N9	3.58 ± 0.40	20	982	16	2		0.020
N10	3.58 ± 0.40	21	980	19	1		0.021
N11	3.58 ± 0.40	17	984	15	1		0.017
N12	3.58 ± 0.40	15	986	13	1		0.015
N13*							
N14	3.58 ± 0.40	24	978	20	2		0.024
N15*							0.017
N=12	**Mean**	**19.7**				**Mean**	**0.020**
	SD	**3.8**				**SD**	**0.004**

*Rats died during experiment due to unknown reason; not able to obtain data.

Table 17. Influence of background radiation on MN frequencies in peripheral lymphocytes of rats exposed to HBNR and NBR sand

Duration (months)	Age (months)	HNBR sand				NBR sand				P value
		Radiation dose (mGy)	N	MN/1000 cells	MN/cell	Radiation dose (mGy)	N	MN/1000 cells	MN/cell	
3	4	23.23 ± 0.97	30	**15.0±2.9**	0.015±0.003	0.60 ± 0.07	15	**15.3±3.0**	0.015±0.003	**0.802**
6	7	46.46 ± 1.95	30	**16.7±4.4**	0.017±0.004	1.19 ± 0.13	15	**16.4±3.9**	0.016±0.004	**0.805**
9	10	69.69 ± 2.92	29	**17.2±4.6**	0.017±0.004	1.79 ± 0.20	15	**16.9±3.7**	0.017±0.004	**0.785**
12	13	92.91 ± 3.89	27	**17.1±4.9**	0.017±0.005	2.38 ± 0.26	14	**17.5±3.8**	0.018±0.004	**0.797**
15	16	116.15 ± 4.86	26	**18.5±5.1**	0.019±0.005	2.98 ± 0.33	14	**17.9±3.7**	0.018±0.004	**0.695**
18	19	139.38 ± 5.84	24	**20.3±4.4**	0.020±0.004	3.58 ± 0.40	12	**19.7±3.8**	0.020±0.004	**0.680**

Table 18. Influence of age on MN frequencies in peripheral lymphocytes of rats exposed to HBNR sand

Parameters	HBNR sand exposed						P value
	Values obtained at different ages (months)						
	4	7	10	13	16	19	
Exposure duration (months)	3	6	9	12	15	18	
Radiation Dose (mGy)	23.23 ± 0.97	46.46 ± 1.95	69.69 ± 2.92	92.91 ± 3.89	116.15 ± 4.86	139.38 ± 5.84	
N	30	30	29	27	26	24	
MN/1000 cells	15.0±2.9	16.7±4.4	17.2±4.6	17.1±4.9	18.5±5.1	20.3±4.4	0.001*
MN/cell	0.015±0.003	0.017±0.004	0.017±0.004	0.017±0.005	0.019±0.005	0.020±0.004	

Table 19. Influence of age on MN frequencies in peripheral lymphocytes of rats exposed to NBR sand

Parameters	NBR sand exposed						P value
	Values obtained at different ages (months)						
	4	7	10	13	16	19	
Exposure duration (months)	3	6	9	12	15	18	
Radiation Dose (mGy)	0.60 ± 0.07	1.19 ± 0.13	1.79 ± 0.20	2.38 ± 0.26	2.98 ± 0.33	3.58 ± 0.40	
N	15	15	15	14	14	12	
MN/1000 cells	15.3±3.0	16.4±3.9	16.9±3.7	17.5±3.8	17.9±3.7	19.7±3.8	0.041*
MN/cell	0.015±0.003	0.016±0.004	0.017±0.004	0.018±0.004	0.018±0.004	0.020±0.004	

*Significant at 0.05 level

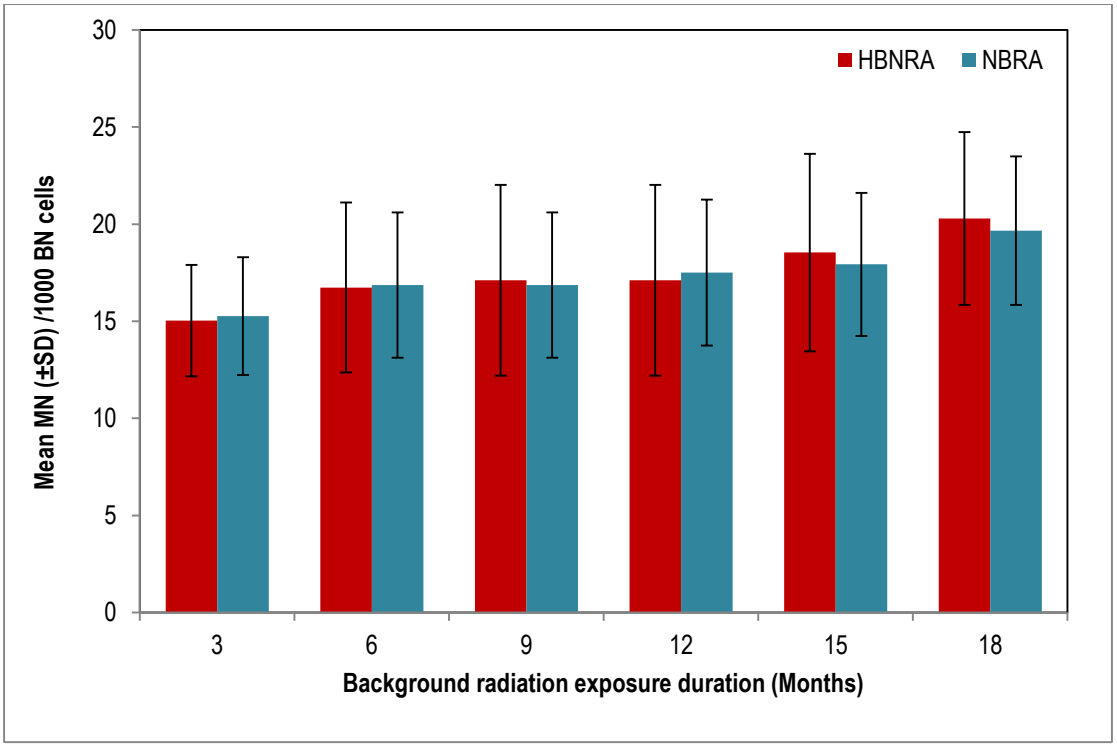


Fig. 5. Influence of background radiation on MN frequencies in rat peripheral lymphocytes.

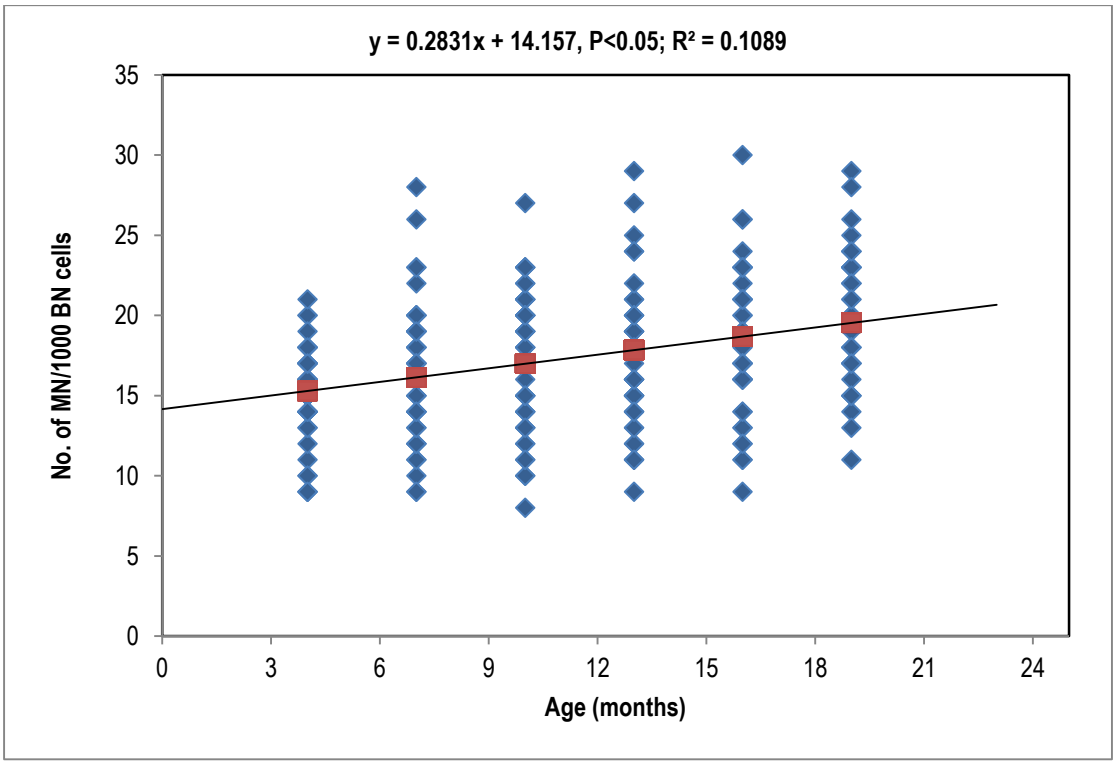


Fig. 6. Influence of age on MN frequencies in rat peripheral lymphocytes.

3.4 Chromosomal aberrations

Chromosomal aberration studies were carried out in Wistar rats. The 30 rats exposed to HBNR sand and 15 rats exposed to NBR sand (control) were studied. Blood samples drawn at an interval of 6 months, up to 18 months, were analyzed for aberrations such as chromosomal gaps, breaks, dicentrics, rings and fragments. The metaphase spread of chromosomes stained using Giemsa's azur-eosin-methylene blue solution were used for analyzing aberrations. The normal rat metaphase spread is shown in Fig. 7.

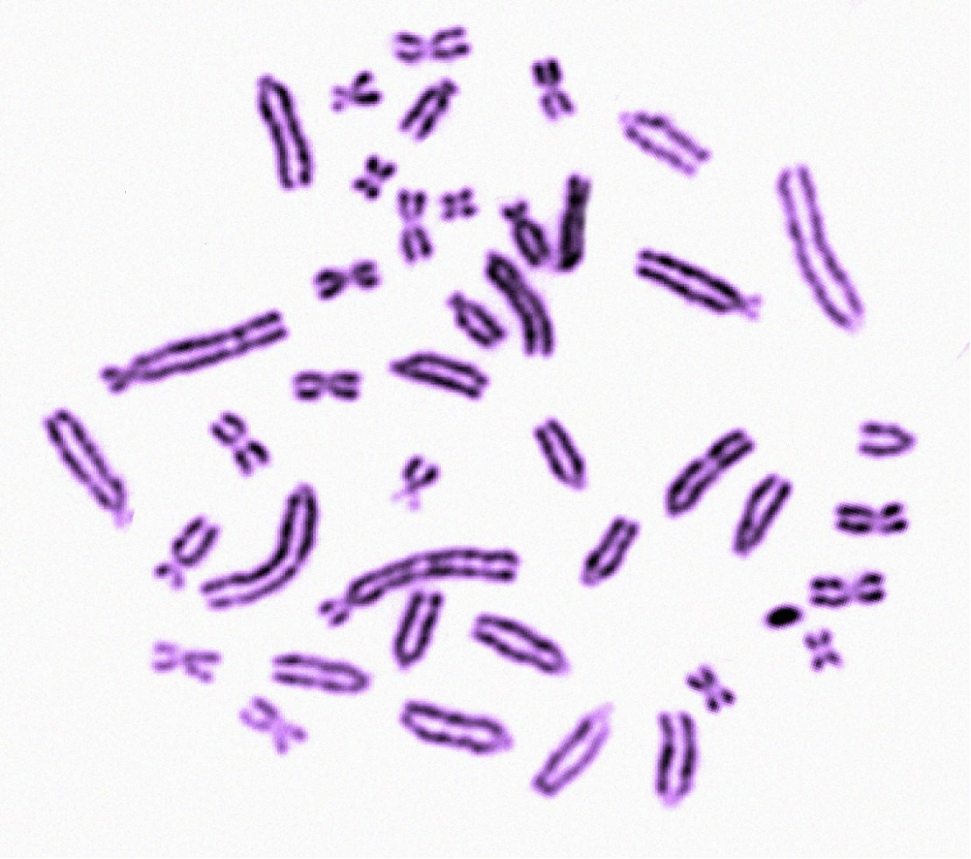


Fig. 7. Normal rat metaphase chromosomes spread without any exposure (Magnification x1000)

Nomenclature of rat chromosomes was prepared based on Committee for Standardized Karyotype of *Rattus norvegicus* (1973). The Giemsa bands of the rat karyotype were described and numbered according to the nomenclature. The diploid

rat chromosomal complement consists of 20 autosomal pairs and the XX or XY sex chromosomes. The chromosome sizes, types, number of G-bands were enumerated (Table 20). The karyotype using G-band technique (Fig. 9) was arranged based on the ideogram (Fig. 8).

Table 20. The rat chromosome sizes, types and number of G-bands

Chromosome Number	Chromosome size (%)[a]	Chromosome size (Mb)[b]	Chromosome type	No. of G bands[c]
1	10.62	267.9	subtelocentric	22
2	9.46	258.2	telocentric	21
3	7.14	171.1	acrocentric[d]	17
4	6.98	187.1	telocentric	15
5	6.38	173.1	telocentric	13
6	5.58	147.6	telocentric	13
7	5.41	143.0	telocentric	11
8	5.02	129.0	telocentric	9
9	4.76	113.4	telocentric	12
10	4.51	110.7	telocentric	10
11	3.87	87.8	subtelocentric	7
12	2.68	46.8	acrocentric[d]	8
13	4.16	111.2	subtelocentric	13
14	4.08	112.2	metacentric	6
15	3.90	109.8	metacentric	13
16	3.55	90.2	metacentric	8
17	3.51	97.3	metacentric	6
18	3.36	87.3	metacentric	6
19	2.70	59.2	metacentric	6
20	2.36	55.3	metacentric	5
X	5.73	160.7	telocentric[e]	13
Y	3.20	UN[f]	telocentric	2

[a] Percent of haploid autosomal set (Committee for a Standardized Karyotype of *Rattus norvegicus,* 1973).
[b] According to the Ensembl database (v37).
[c] Nomenclature for G-bands in Rat Chromosomes (Levan, 1974).
[d] Nucleolar organizing (NOR) chromosomes with satellites at the short arm.
[e] In some inbred rat strains the X chromosome is subtelocentric.
[f] UN: unknown

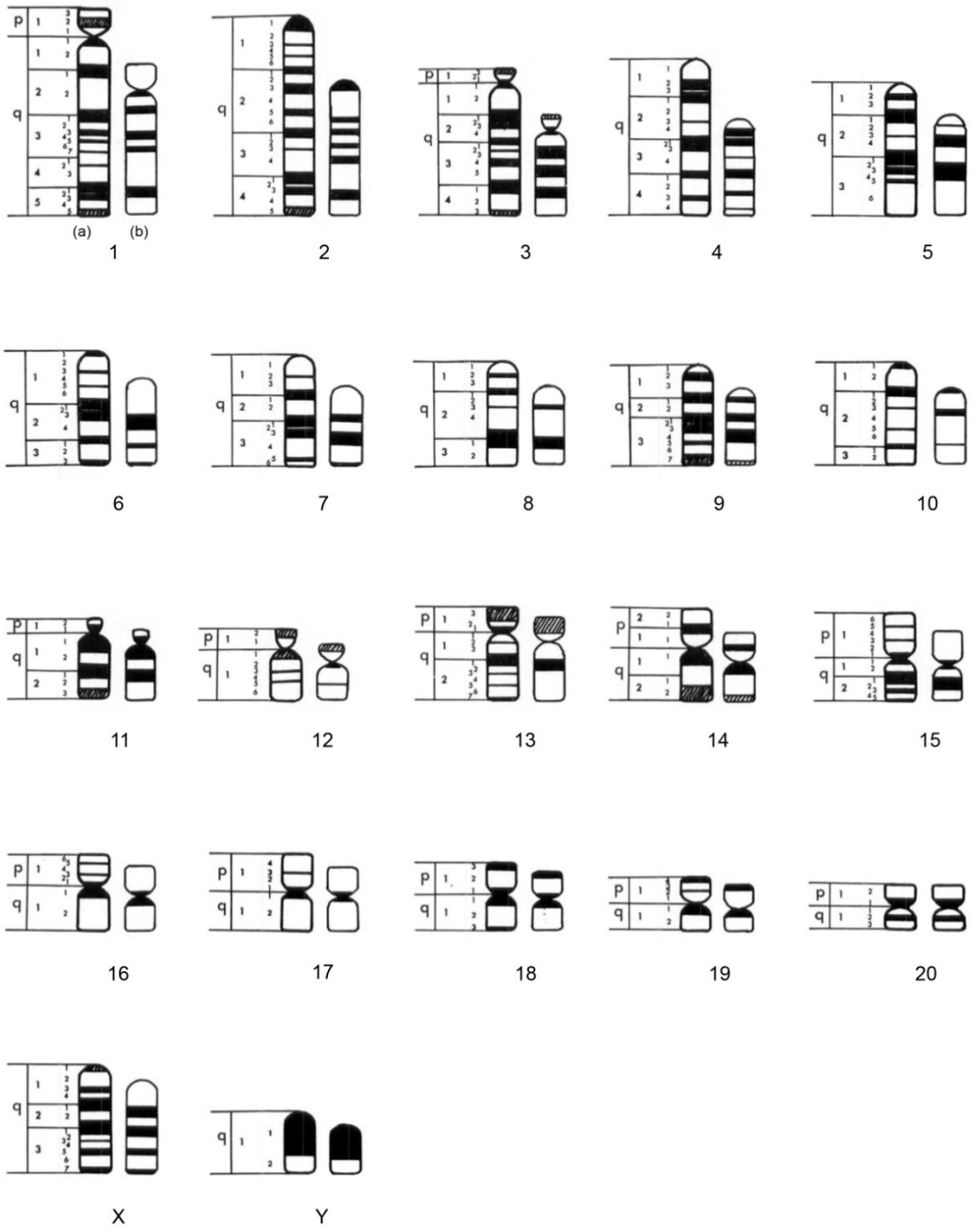


Fig. 8. Ideogram showing the number and types of chromosomes, (a) less contracted patterns (b) more contracted patterns (Source: Levan, 1974)

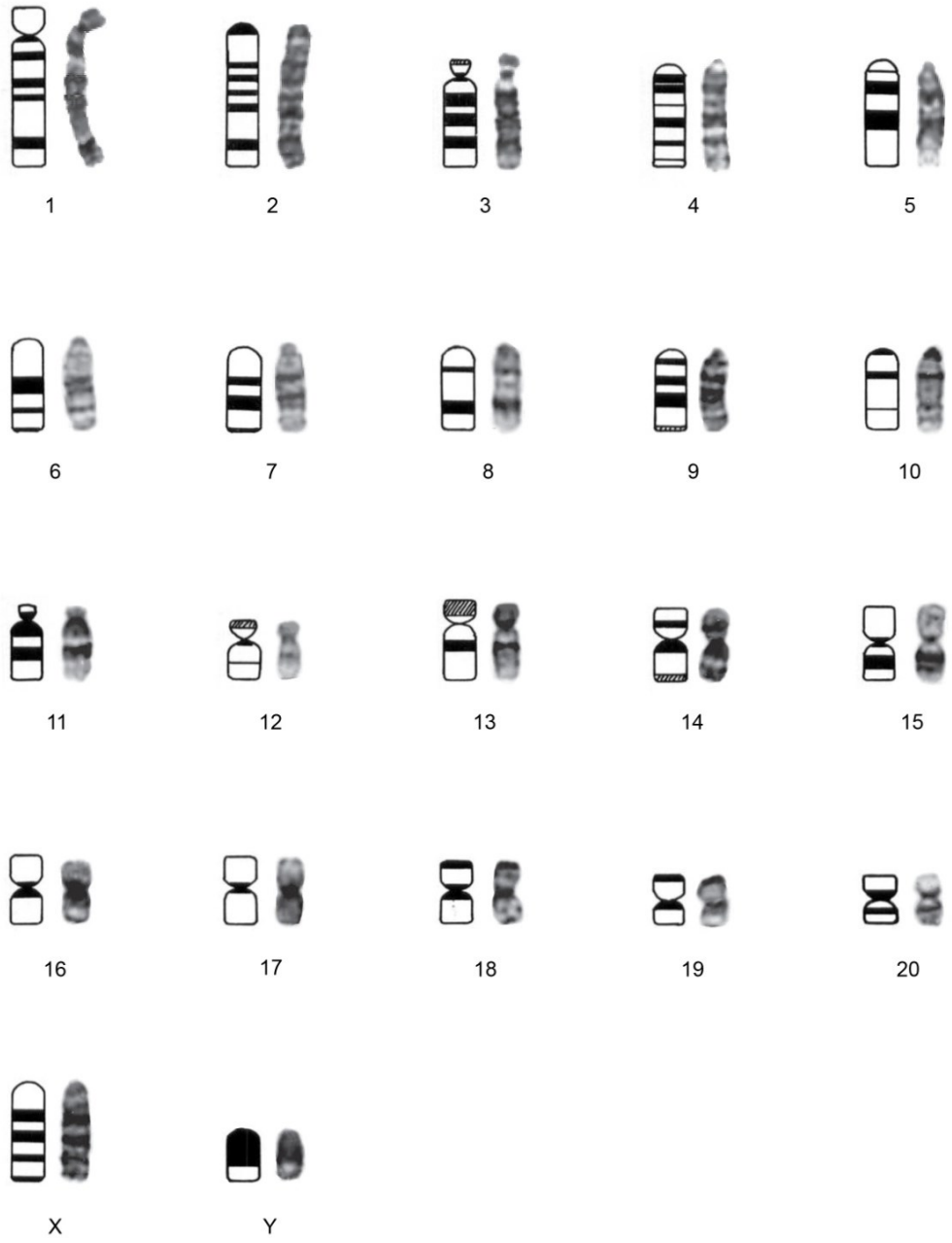


Fig. 9. Karyotyping of rat chromosomes by G-banding technique

Distribution of CAs in HBNR and NBR sand-exposed rats at different time intervals were enumerated and shown in Tables 21 to 26. CAs per 100 cells were calculated at each time intervals in both HBNR and NBR sand-exposed rats. In HBNR rats it was found to be 3.6±1.7, 4.5±1.4, and 6.1±1.7 per 100 metaphase spreads, at the dose rate of 46.46±1.95 mGy (6 months), 92.91±3.89 mGy (12 months) and 139.38±5.84 mGy (18 months), respectively, whereas it was 3.7±1.4, 4.4±1.7 and 5.9±1.8 per 100 metaphase spreads from NBR sand-exposed rats at the dose rate of 1.19 ± 0.13 mGy (6 months), 2.38 ± 0.26 mGy (12 months) and 3.58 ± 0.40 mGy (18 months), respectively (Table 27, Fig. 11). No statistical difference was found in CAs between HBNR and NBR sand-exposed rats using ANOVA for 6 months [$F(1,43) = 0.013$, $P > 0.05$], 12 months [$F(1,39) = 0.097$, $P > 0.05$], and 18 months [$F(1,34) = 0.124$, $P > 0.05$]. As is the case with the MN frequency, this too might be due to the fact that the level of radiation exposure was too low to induce significant CAs. On the other hand, it is also possible that the damage that had been induced significantly, was repaired. In the present study structural CAs such as dicentrics, rings and fragments were analyzed (Fig. 10), which are unstable aberrations and can be repaired. The stable structural CAs such as deletions, translocation, insertion, inversion and isochromosomes were not analyzed. However, an age-dependent increase in the frequency of CA was observed (Table 26). Analysis of variance suggested that the difference in frequencies was statistically significant among both HBNR and NBR sand-exposed age groups [$F(2,78) = 16.044$, $P < 0.05$ and $F(2,38) = 6.044$, $P < 0.05$, respectively]. *Post-hoc* analysis of CA in the age groups in both HBNR and NBR sand-exposed revealed that the mean difference was significant at the 0.05 level. Linear regression analysis showed a significant increase in the CA with respect to age [$Y= 0.1954X + 2.1879$; $R^2 = 0.2662$, $P < 0.05$ (Fig. 12)]. The age-related increase in CA in rats was comparable in HBNR and NBR sand-exposed rats.

The percentage-wise distribution of CA showed that the higher proportion of aberrations were breaks (56.2%, 60.1% and 59.0%) followed by gaps (27.3%, 26.8% and 26.1%), dicentrics (8.3%, 6.5% and 7.5%), fragments (4.1%, 3.6% and 5.6%) and rings (4.1%, 2.9% and 1.9%) in HBNR sand-exposed rats at the dose of 46.46±1.95 mGy (6 months), 92.91±3.89 mGy (12 months) and 139.38±5.84 mGy (18 months), respectively (Fig. 13). In NBR sand-exposed rats also the higher proportion of aberrations were breaks (61.3%, 61.2% and 72.2%) followed by gaps (25.8%, 28.4% and 20.3%), dicentrics (6.5%, 4.5% and 3.8%), fragments (4.8%, 4.5% and 2.5%) and rings (1.6%, 1.5% and 1.3%) at the dose of 1.19 ± 0.13 mGy (6 months), 2.38 ± 0.26 mGy (12 months) and 3.58 ± 0.40 mGy (18 months), respectively (Fig. 14).

Ionizing radiation is known to induce DNA double-strand breaks (DSBs) and the mis-repair of these DSBs might lead to CAs like dicentrics, rings, translocations, inversions, fragments and breaks (Natarajan, 1993; Pfeiffer *et al*., 2000). In addition, some other studies in HBNR areas showed significantly higher number of CAs compared to control areas. The individuals from Ramsar, Iran were found to have a significantly higher frequency of unstable CAs (mainly breaks) in HBNRA as compared to controls (Ghiassi-Nejad *et al*., 2004). A study in Brazil reported a significant increase in total chromosomal breaks in individuals residing in HBNRA, when compared to those residing in a control area (Barcinski *et al*., 1975). On the contrary, the frequency of unstable aberrations in rats exposed to HBNR and NBR sand in the present study was not statistically significant, and frequency of unstable aberrations were observed to be comparable in HBNR and NBR sand-exposed groups. No dose response was observed in the different background dose groups. Some other studies conducted in high background radiation areas and control areas support the findings of the present study. Genetic monitoring of the human population – in 8,493

newborns residing in high-level natural radiation areas (dose rate >1.5 mGy/year) and 1,737 newborns residing in normal-level natural radiation areas (≤1.5 mGy/year) – did not show any significant difference in the frequency of dicentrics, or other types of aberrations known to be associated with radiation exposure (Cheriyan *et al*., 1999). A recent study of residents of high- and normal-level natural radiation areas of Kerala showed no statistically significant difference in frequency of unstable aberrations, which was lower in HBNRA especially among individuals exposed to a dose of >5 mGy/year (Karuppasamy *et al*., 2018). This could be due to the effect of adaptive response and *in vivo* studies show the efficient repair of DSBs in individuals residing in HBNRAs (Kumar *et al*., 2005; Ramachandran *et al*., 2017; Jain *et al*., 2017). The possible reason for the difference in results for unstable aberrations in this study when compared to other studies from high levels of natural radiation areas of the world could be due to other factors including study design, confounding factors, other environmental exposures, level of radiation exposure and adaptive response.

Influences of age on the CA yield are quite inconsistent. Poisson regression analysis was carried out in residents of high- and normal-level natural radiation areas of Kerala, which indicated no age-related increase in CAs (Karuppasamy *et al*., 2018). However, other investigators have reported effect of age on the yield of CAs (Tucker *et al*., 1994; Ramsey *et al*., 1995). A significant increase in translocation yield with respect to age was reported in individuals by using FISH (Pressl *et al*., 1999). The positive correlation of unstable aberrations with respect to age of the subjects was observed in high background radiation areas of China, which was attributed to cumulative radiation dose, but no increase was observed in control areas (Jiang *et al*., 2000). The present study showed age-dependent increase in unstable CAs in rats exposed to both HBNR and NBR sands.

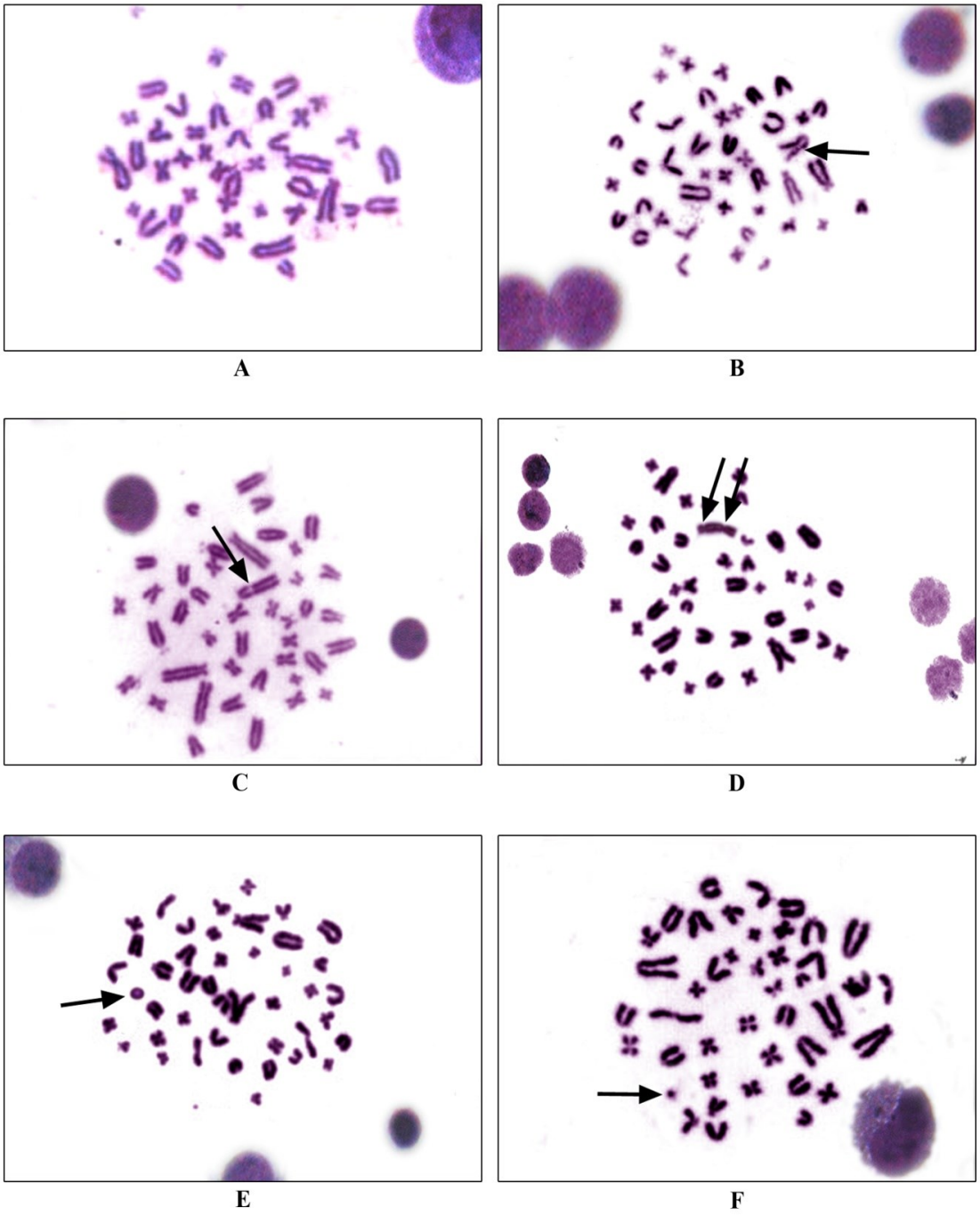


Fig. 10. Different types of chromosomal aberrations in rat peripheral lymphocytes. A) metaphase image showing normal chromosomes, B) metaphase image showing chromosomal gap, C) metaphase image showing chromosomal break, D) metaphase image showing dicentrics, E) metaphase image showing ring chromosome, and F) metaphase image showing chromosomal fragment. 2n=42 (Magnification x400)

Table 21. Distribution of CAs in peripheral lymphocytes of 6-month HBNR sand-exposed rats

Sample code	Radiation dose (mGy)	Mitotic index	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells
			Gaps	Breaks	Dicentrics	Rings	Fragments		
H1	46.46 ± 1.95	10.3	1	2				2.5	2.5
H2	46.46 ± 1.95	9.8	2	2	1	1		4.3	5.1
H3	46.46 ± 1.95	10.1	1	2				3.5	2.6
H4	46.46 ± 1.95	10.3		3				1.7	2.5
H5	46.46 ± 1.95	9.1	1	4	1		1	5.9	6.9
H6	46.46 ± 1.95	9.6		4				2.8	3.7
H7	46.46 ± 1.95	9.9	2	3				4.5	4.5
H8	46.46 ± 1.95	9.6		2				0.9	1.7
H9	46.46 ± 1.95	8.7	1	2				2.0	2.9
H10	46.46 ± 1.95	9.4	1	3	1	1		3.8	5.7
H11	46.46 ± 1.95	10.1		2	1		1	2.6	3.5
H12	46.46 ± 1.95	10.0	2	3				4.2	4.2
H13	46.46 ± 1.95	8.9		2				1.0	1.9
H14	46.46 ± 1.95	10.3		2				0.9	1.7
H15	46.46 ± 1.95	9.5	1	1				2.8	1.8
H16	46.46 ± 1.95	9.1	2	1	2	1	1	4.9	6.8
H17	46.46 ± 1.95	10.1	2	4				5.2	5.2
H18	46.46 ± 1.95	9.9	3	3				5.4	5.4
H19	46.46 ± 1.95	10.5	1	2	1	1		3.3	4.2
H20	46.46 ± 1.95	10.3	1	2				2.6	2.6
H21	46.46 ± 1.95	10.3	1	1				1.7	1.7
H22	46.46 ± 1.95	8.8	2					2.0	2.0
H23	46.46 ± 1.95	9.1	2	1				2.8	2.8
H24	46.46 ± 1.95	9.0	3	3				4.7	5.7
H25	46.46 ± 1.95	9.7		3	1		1	3.4	4.3
H26	46.46 ± 1.95	9.0	1					1.0	1.0
H27	46.46 ± 1.95	9.3	1	2	1		1	3.8	4.8
H28	46.46 ± 1.95	10.3		2				1.7	1.7
H29	46.46 ± 1.95	10.1	2	4				4.2	5.1
H30	46.46 ± 1.95	8.7		3	1	1		3.0	5.0
N=30	**Mean**	**9.7**						**Mean**	**3.6**
	SD	**0.6**						**SD**	**1.7**

Table 22. Distribution of CAs in peripheral lymphocytes of 6-month NBR sand-exposed rats

Sample code	Radiation dose (mGy)	Mitotic index	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells
			Gaps	Breaks	Dicentrics	Rings	Fragments		
N1	1.19 ± 0.13	9.7	2	2				2.7	3.6
N2	1.19 ± 0.13	9.7	1	3	1		1	4.4	5.3
N3	1.19 ± 0.13	9.3	2	2				3.8	3.8
N4	1.19 ± 0.13	9.3		3				1.8	2.7
N5	1.19 ± 0.13	10.6		3				2.5	2.5
N6	1.19 ± 0.13	10.2		2				0.9	1.7
N7	1.19 ± 0.13	10.1	1	3	1	1		3.5	5.3
N8	1.19 ± 0.13	9.3	1	3				2.8	3.7
N9	1.19 ± 0.13	10.4	2	2				2.5	3.4
N10	1.19 ± 0.13	9.7	1	4				1.8	4.5
N11	1.19 ± 0.13	9.9		3	1		1	3.4	4.2
N12	1.19 ± 0.13	9.4	2	3	1		1	4.8	6.7
N13	1.19 ± 0.13	9.3	1	3				2.8	3.7
N14	1.19 ± 0.13	9.8	1	1				1.7	1.7
N15	1.19 ± 0.13	9.2	2	1				2.8	2.8
N=15	**Mean**	**9.7**						**Mean**	**3.7**
	SD	**0.4**						**SD**	**1.4**

Table 23. Distribution of CAs in peripheral lymphocytes of 12-month HBNR sand-exposed rats.

Sample code	Radiation dose (mGy)	Mitotic index	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells
			Gaps	Breaks	Dicentrics	Rings	Fragments		
H1	92.91 ± 3.89	9.9	2	2	1	1		4.5	5.4
H2	92.91 ± 3.89	9.5	1	3				3.7	3.7
H3	92.91 ± 3.89	9.1	2	4				5.7	5.7
H4	92.91 ± 3.89	11.0		3				2.4	2.4
H5	92.91 ± 3.89	9.7	2	4				4.5	5.4
H6	92.91 ± 3.89	9.3	2	2				3.7	3.7
H7	92.91 ± 3.89	9.8	1	3	2	1	1	5.1	6.8
H8	92.91 ± 3.89	10.0	2	2	1		1	4.2	5.0
H9*									
H10	92.91 ± 3.89	10.0	2	5				6.2	6.2
H11	92.91 ± 3.89	9.7	2	3				3.7	4.6
H12	92.91 ± 3.89	8.8	2	1				3.0	3.0
H13*									
H14	92.91 ± 3.89	9.5	2	2				3.6	3.6
H15	92.91 ± 3.89	10.3	2	3				3.3	4.1
H16	92.91 ± 3.89	10.3	1	5	1		1	5.1	6.8
H17	92.91 ± 3.89	10.7		5				4.0	4.0
H18	92.91 ± 3.89	10.0	1	4				4.4	4.4
H19	92.91 ± 3.89	9.9	3		1	1		3.5	4.3
H20	92.91 ± 3.89	9.6		2				1.8	1.8
H21*									
H22	92.91 ± 3.89	10.0	1	4				4.2	4.2
H23	92.91 ± 3.89	9.3		3				2.9	2.9
H24	92.91 ± 3.89	9.8		3				2.7	2.7
H25	92.91 ± 3.89	9.0	2	4				4.8	5.8
H26	92.91 ± 3.89	8.7	2	1	1		1	3.9	4.9
H27	92.91 ± 3.89	9.7	1	5				4.6	5.6
H28	92.91 ± 3.89	9.5	2	3	1	1		4.7	6.5
H29	92.91 ± 3.89	10.1	1	4	1		1	5.2	6.0
H30	92.91 ± 3.89	10.5	1	3				3.3	3.3
N=27	**Mean**	**9.8**						**Mean**	**4.5**
	SD	**0.5**						**SD**	**1.4**
*Rats died during experiment due to unknown reason; not able to obtain data.									

Table 24. Distribution of CAs in peripheral lymphocytes of 12-months NBR sand-exposed rats.

Sample code	Radiation dose (mGy)	Mitotic index	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells
			Gaps	Breaks	Dicentrics	Rings	Fragments		
N1	2.38 ± 0.26	10.2	2	2				3.4	3.4
N2	2.38 ± 0.26	8.7	2	4	1		2	6.9	8.8
N3	2.38 ± 0.26	9.7	2	3				4.6	4.6
N4*									
N5	2.38 ± 0.26	8.8	1	2				3.0	3.0
N6	2.38 ± 0.26	9.8	2	2	1	1		4.4	5.3
N7	2.38 ± 0.26	8.6	1	3				4.0	4.0
N8	2.38 ± 0.26	9.9	2	4				5.0	5.0
N9	2.38 ± 0.26	9.4	2	3				4.6	4.6
N10	2.38 ± 0.26	9.0	1	4	1		1	5.9	6.9
N11	2.38 ± 0.26	9.5	1	2				2.7	2.7
N12	2.38 ± 0.26	10.1		4				3.5	3.5
N13	2.38 ± 0.26	10.4	2	3				4.3	4.3
N14	2.38 ± 0.26	9.9		3				2.6	2.6
N15	2.38 ± 0.26	9.5	1	2				2.8	2.8
N=14	**Mean**	**9.5**						**Mean**	**4.4**
	SD	**0.6**						**SD**	**1.7**

*Rats died during experiment due to unknown reason; not able to obtain data.

Table 25. Distribution of CAs in peripheral lymphocytes of 18-month HBNR sand-exposed rats.

Sample code	Radiation dose (mGy)	Mitotic index	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells
			Gaps	Breaks	Dicentrics	Rings	Fragments		
H1*									
H2	139.38 ± 5.84	9.1	2	4				5.9	5.9
H3*									
H4	139.38 ± 5.84	10.1	1	5	1		1	4.3	6.8
H5	139.38 ± 5.84	10.6	2	5				5.6	5.6
H6	139.38 ± 5.84	9.3	1	5	1		1	5.8	7.8
H7	139.38 ± 5.84	9.6	3	2				4.6	4.6
H8	139.38 ± 5.84	9.9	2	4	1	1		6.0	6.9
H9*									
H10	139.38 ± 5.84	9.5	3	4	2		2	7.1	9.8
H11	139.38 ± 5.84	9.8	2	2				3.5	3.5
H12	139.38 ± 5.84	9.0	2	3				3.7	4.6
H13*									
H14*									
H15	139.38 ± 5.84	9.1		4	1		1	4.8	5.8
H16	139.38 ± 5.84	10.0	2	5				6.2	6.2
H17	139.38 ± 5.84	9.6	3	4	1		1	7.5	8.4
H18	139.38 ± 5.84	10.6	2	5				5.8	5.8
H19	139.38 ± 5.84	9.8	1	4	1	1		5.4	6.3
H20	139.38 ± 5.84	9.0	3	4				4.9	6.8
H21*									
H22	139.38 ± 5.84	10.1		4				3.4	3.4
H23	139.38 ± 5.84	9.3	1	5				4.5	5.5
H24	139.38 ± 5.84	8.8	2	4	1		1	6.9	7.9
H25	139.38 ± 5.84	9.0	3	4				5.7	6.7
H26	139.38 ± 5.84	8.4	2	2	1		1	5.0	6.0
H27	139.38 ± 5.84	9.5	2	4	2	1	1	7.3	9.2
H28	139.38 ± 5.84	9.4	2	3				4.7	4.7
H29	139.38 ± 5.84	10.4		6				5.0	5.0
H30	139.38 ± 5.84	10.3	1	3				3.4	3.4
N=24	**Mean**	**9.6**						**Mean**	**6.1**
	SD	**0.6**						**SD**	**1.7**
*Rats died during experiment due to unknown reason; not able to obtain data.									

Table 26. Distribution of CAs in peripheral lymphocytes of 18-month NBR sand-exposed rats.

Sample code	Radiation dose (mGy)	Mitotic index	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells
			Gaps	Breaks	Dicentrics	Rings	Fragments		
N1	3.58 ± 0.40	9.7	2	3				4.4	4.4
N2	3.58 ± 0.40	9.0		7	1	1		6.6	8.5
N3	3.58 ± 0.40	10.0	1	4				3.4	4.2
N4*									
N5	3.58 ± 0.40	9.1	2	3				4.0	5.0
N6	3.58 ± 0.40	9.7	1	6				5.5	6.4
N7	3.58 ± 0.40	9.8	2	7	1		1	6.8	9.3
N8	3.58 ± 0.40	9.6	1	4				4.5	4.5
N9	3.58 ± 0.40	9.8	1	4	1		1	5.2	6.0
N10	3.58 ± 0.40	9.2		8				5.5	7.3
N11	3.58 ± 0.40	9.0	1	3				3.9	3.9
N12	3.58 ± 0.40	9.8	3	5				6.1	7.0
N13*									
N14	3.58 ± 0.40	10.1	2	3				3.4	4.2
N15*									
N=12	**Mean**	**9.6**						**Mean**	**5.9**
	SD	**0.4**						**SD**	**1.8**
*Rats died during experiment due to unknown reason; not able to obtain data.									

Table 27. Influence of background radiation on CAs in peripheral lymphocytes of rats exposed to HBNR and NBR sand

Exposure duration (Months)	Type of exposure	Radiation dose (mGy)	N	Total number of cells	No. of Metaphase	Mitotic index	No. of Abnormal metaphase	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells	P value
								Gaps	Breaks	Dicentrics	Rings	Fragments			
6	HBNR	46.46±1.95	30	31244	3340	9.7±0.6	103	33	68	10	5	5	3.1	**3.6±1.7**	**0.910**
	NBR	1.19±0.13	15	15665	1687	9.7±0.4	47	16	38	4	1	3	2.8	**3.7±1.4**	
12	HBNR	92.91±3.89	27	28061	3039	9.8±0.5	122	37	83	9	4	5	4.0	**4.5±1.4**	**0.757**
	NBR	2.38±0.26	14	14606	1538	9.5±0.6	63	19	41	3	1	3	4.1	**4.4±1.7**	
18	HBNR	139.38±5.84	24	24978	2652	9.6±0.6	140	42	95	12	3	9	5.3	**6.1±1.7**	**0.727**
	NBR	3.58±0.40	12	12656	1339	9.6±0.4	66	16	57	3	1	2	4.9	**5.9±1.8**	

Table 28. Influence of age on CAs in peripheral lymphocytes of rats exposed to HBNR and NBR sand

Source	Age (Months)	Radiation dose (mGy)	N	Total number of cells	No. of Metaphase	Mitotic index	No. of Abnormal metaphase	Chromosomal aberrations					Abnormal metaphase (%)	CA/100 cells	P value
								Gaps	Breaks	Dicentrics	Rings	Fragments			
HBNR sand exposed	7	46.46±1.95	30	31244	3340	9.7±0.6	103	33	68	10	5	5	3.1	3.6±1.7	**0.000***
	13	92.91±3.89	27	28061	3039	9.8±0.5	122	37	83	9	4	5	4.0	4.5±1.4	
	19	139.38±5.84	24	24978	2652	9.6±0.6	140	42	95	12	3	9	5.3	6.1±1.7	
NBR sand exposed	7	1.19±0.13	15	15665	1687	9.7±0.4	47	16	38	4	1	3	2.8	3.7±1.4	**0.005***
	13	2.38±0.26	14	14606	1538	9.5±0.6	63	19	41	3	1	3	4.1	4.4±1.7	
	19	3.58±0.40	12	12656	1339	9.6±0.4	66	16	57	3	1	2	4.9	5.9±1.8	

*Significant at 0.05 level

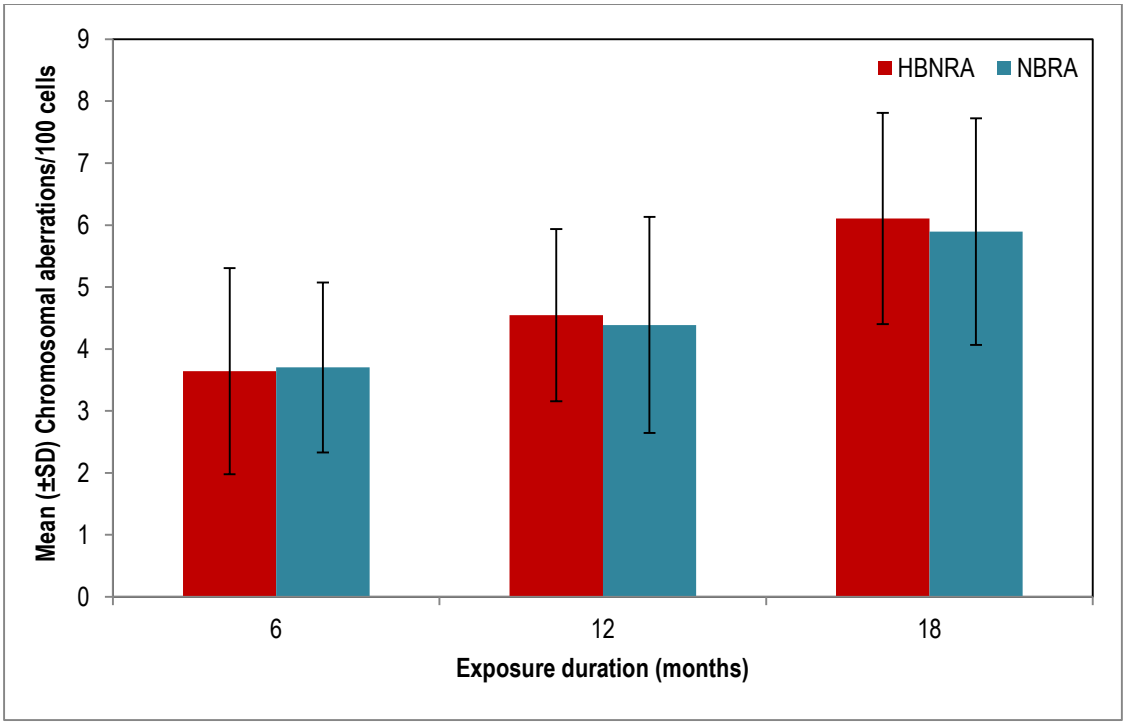


Fig. 11. Influence of background radiation on CAs in rat peripheral lymphocytes.

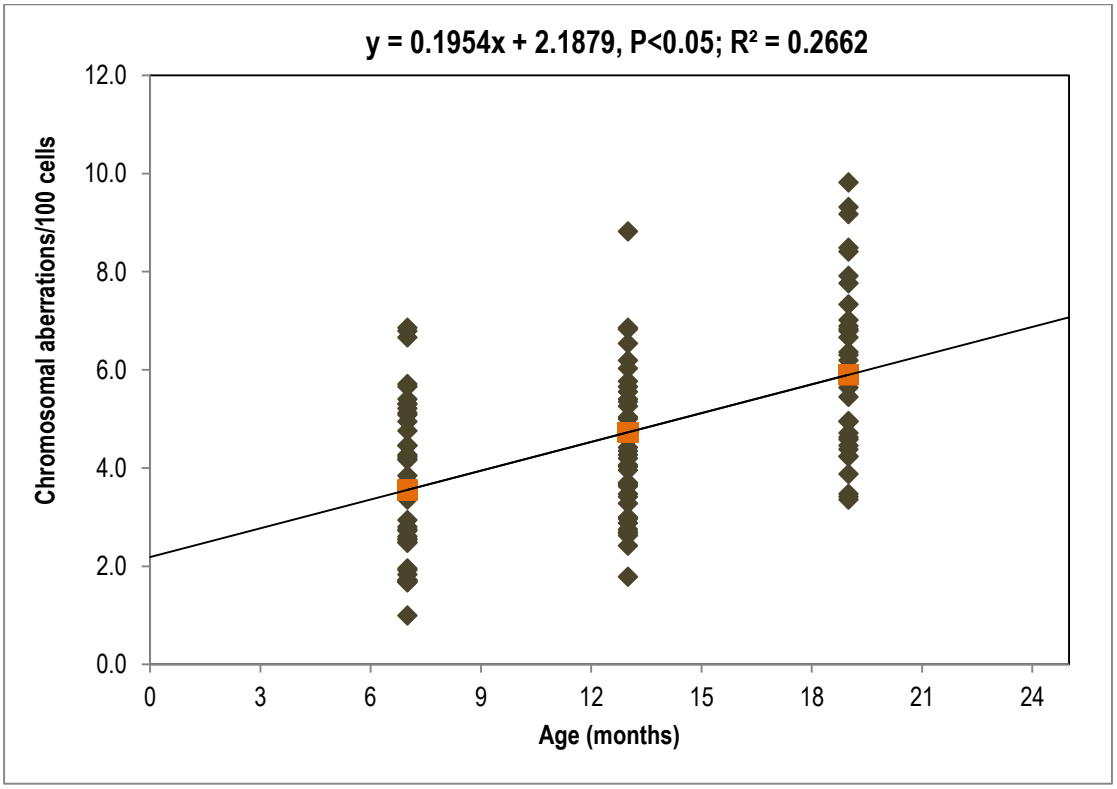


Fig. 12. Influence of age on CAs in rat peripheral lymphocytes

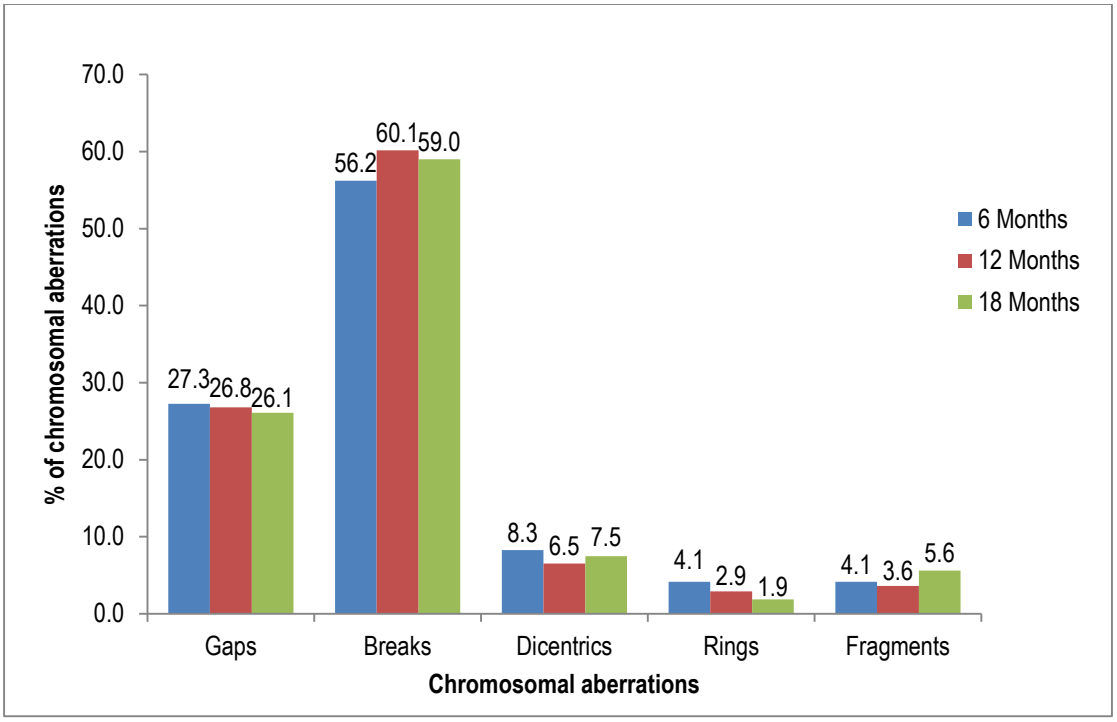


Fig. 13. Percentage of CAs in peripheral lymphocytes of rats exposed to HBNR sand bed at different time intervals.

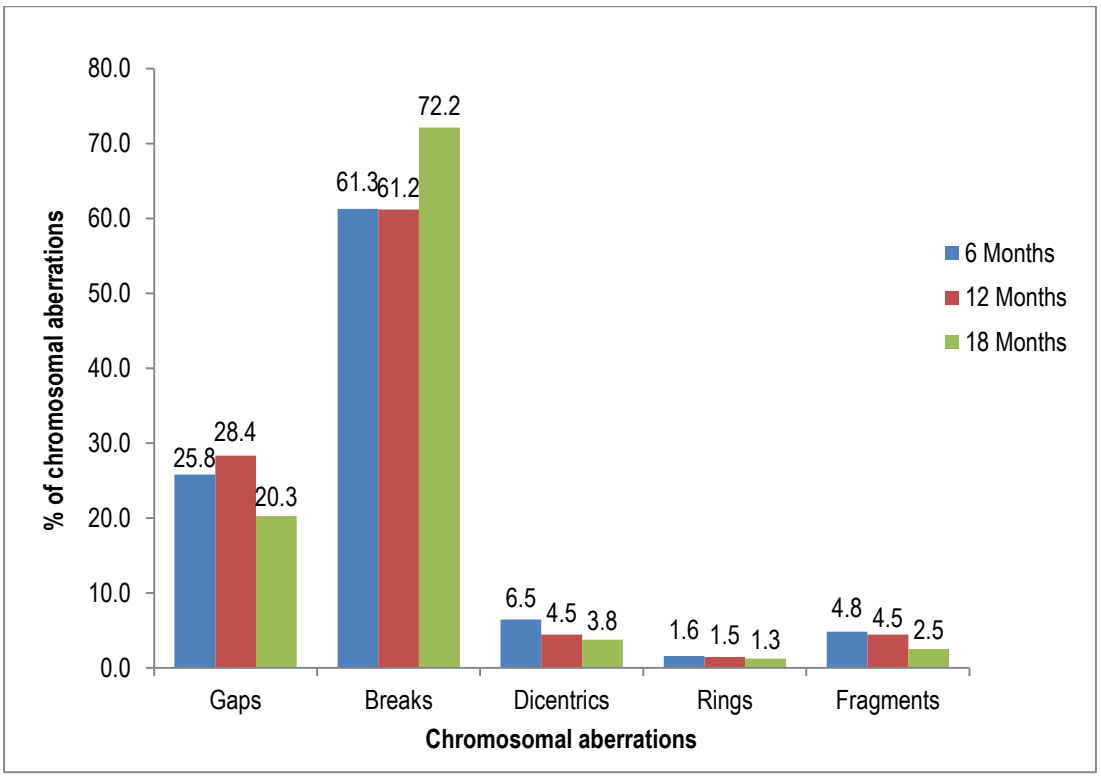


Fig. 14. Percentage of CAs in peripheral lymphocytes of rats exposed to NBR sand bed at different time frames.

3.5 Comet assay

Ten and five rats exposed to HBNR and NBR sand, respectively, were considered for comet assay. Blood samples drawn after 9 and 18 months of exposure were used for comet assay analysis (Figs. 15 and 16). The DNA damage of the cell was primarily assessed by tail DNA%, tail length and tail moment. Since tail moment parameter is widely employed in environmental and occupational studies (Valverde and Rojas, 2009), it was used for the statistical analyses in the present study. Comet assay in HBNR and NBR sand-exposed rats for 9 and 18 months are detailed from Tables 29 to 32. In HBNR rat blood samples, tail moment (μm) was found to be 3.2±1.3 and 3.7±2.0, at the dose rate of 69.69±2.92 mGy (9 months) and 139.38±5.84 mGy (18 months), respectively, whereas it was 3.3±1.8, and 3.4±1.7 from NBR sand-exposed rats at the dose rate of 1.79 ± 0.20 mGy (9 months) and 3.58 ± 0.40 mGy (18 months), respectively (Fig. 17). ANOVA did not show statistical difference in tail moment between HBNR and NBR sand-exposed rats at 9 months [$F(1,13) = 0.007$, $P > 0.05$] and 18 months [$F(1,11) = 0.059$, $P > 0.05$] (Table 33). In HBNR, tail DNA% was found to be 6.3±1.6 and 6.7±1.9, at the dose rate of 69.69±2.92 mGy (9 months) and 139.38±5.84 mGy (18 months), respectively, whereas it was 6.6±1.9 and 6.3±1.9 from NBR sand-exposed rats at the dose rate of 1.79 ± 0.20 mGy (9 months) and 3.58 ± 0.40 mGy (18 months), respectively (Fig. 18).

In the present analysis, no significant DNA damage was found in peripheral blood mononuclear cells (PBMCs) of rats exposed to HBNR sand compared to rats exposed to NBR sand. Kumar *et al*. (2011) too reported no significant difference in basal DNA damage in individuals from HBNRA of Kerala compared to individuals from the NBRA. It was also noted that the elderly individuals from HBNRA showed relatively lower basal DNA damage compared those residing in NBRA. The low basal

damage, as measured in elderly subjects from HBNRA, may be the result of a high efficiency of repair (Wojewódzka *et al*., 1999; Somorovska *et al*., 1999). In contrast to present results, Masoomi *et al*. (2006) reported significantly elevated spontaneous DNA damage in subjects from the HBNRA of Ramsar, Iran, over the control group. Moreover, another study of the Ramsar population recorded higher DNA damage in subjects from HBNRA (Mohammadi *et al*., 2006). The discrepancy in the results seen in this study and from Ramsar could be due in part to the differences in the exposure conditions. Ghiassi-Nejad *et al*. (2002) reported an annual effective dose up to 260 mGy/year, whereas the annual effective dose in HBNR sand bed was up to 92.91±2.38 mGy/year. Another possible reason could be the inter-specific differences, i.e. most of the studies involved human subjects residing in high background natural radiation areas in different parts of the world, whereas, in this study, Wistar rats were used as the experimental model. However, present findings need more validation in a larger group for a longer duration.

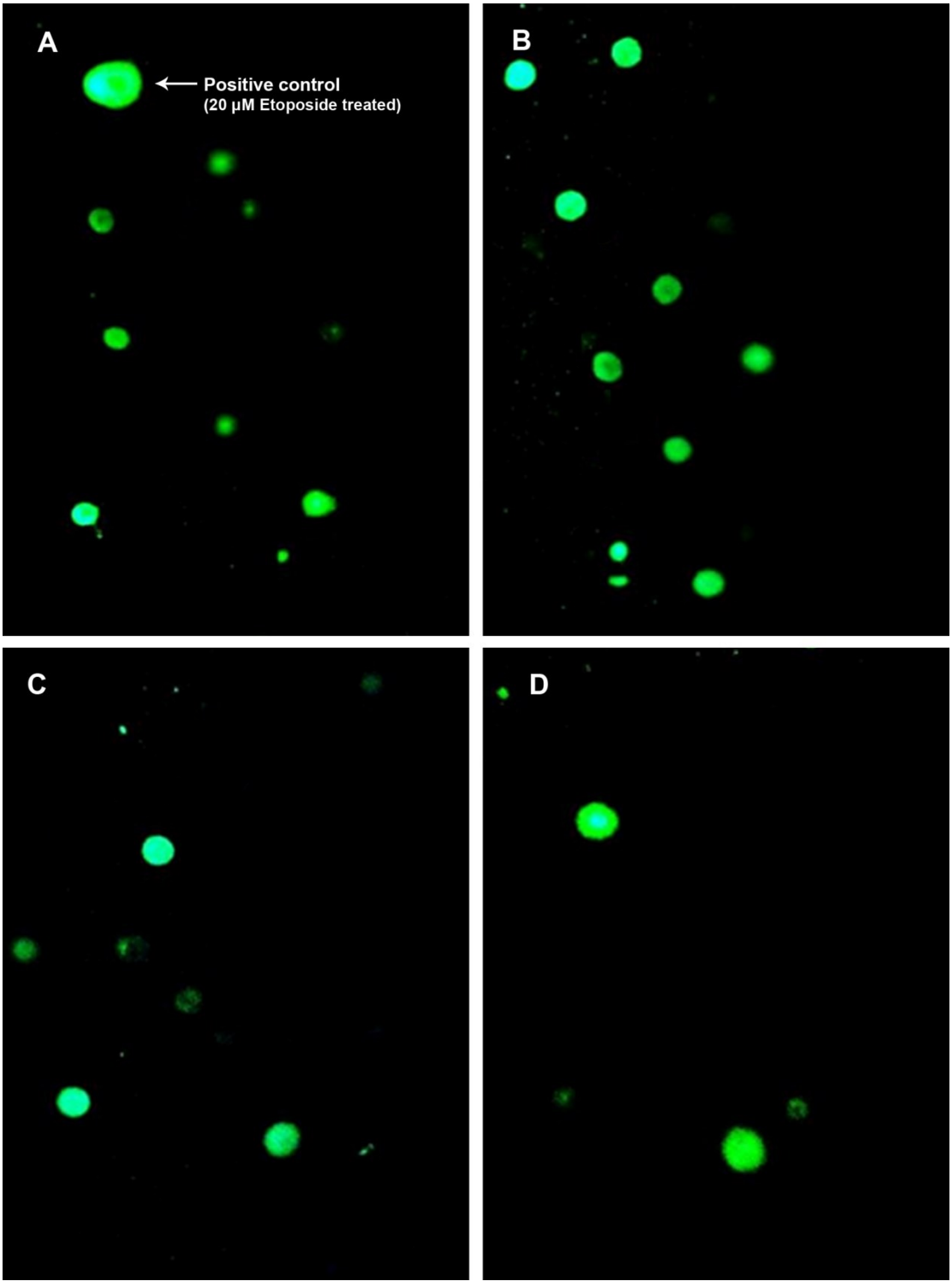


Fig. 15. DNA damage analysis using comet assay in 9-month HBNR sand-exposed rats (A and B) and 9-month NBR sand-exposed rats (C and D)

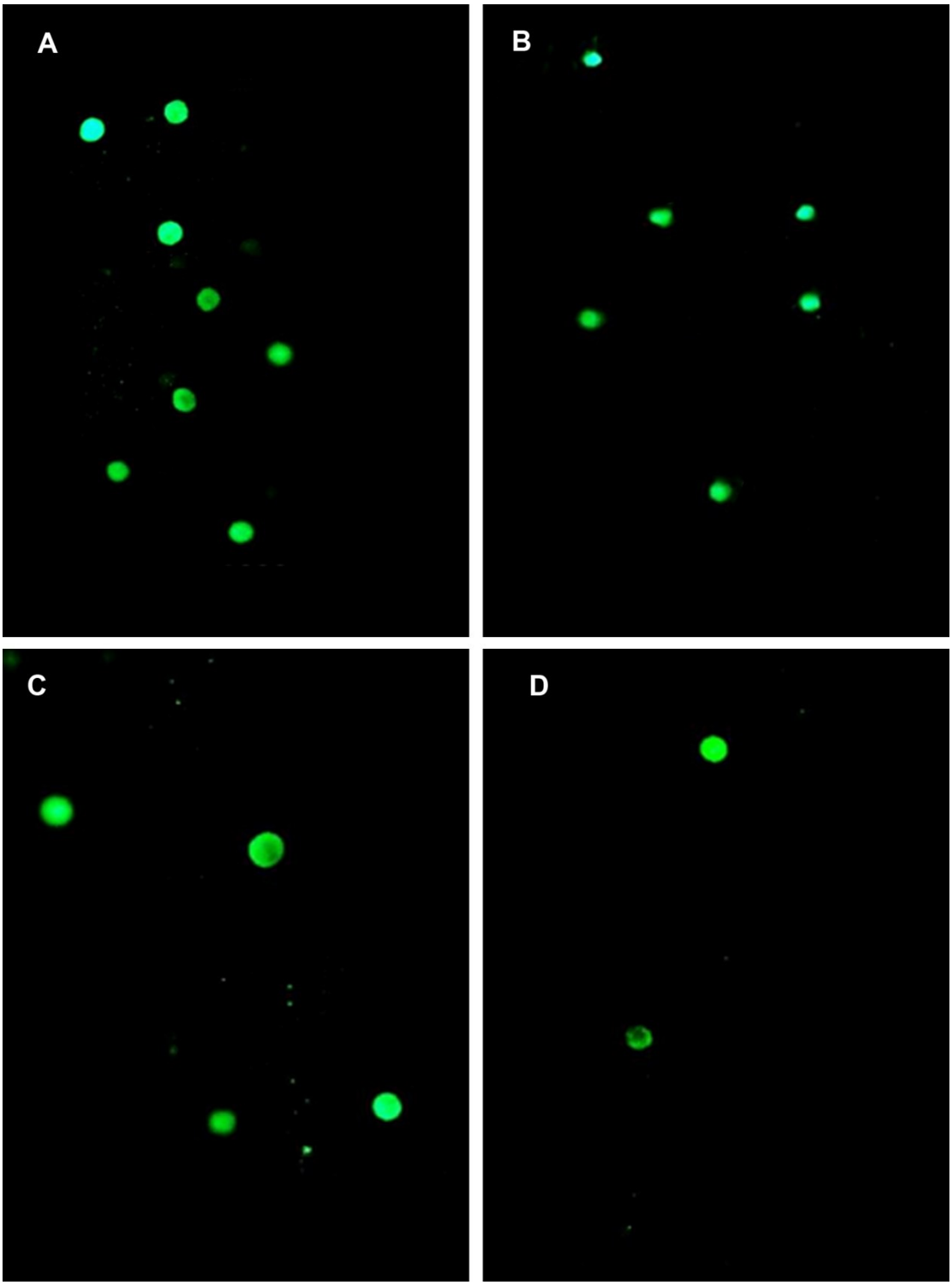


Fig. 16. DNA damage analysis using comet assay in 18-month HBNR sand-exposed rats (A and B) and 18-month NBR sand-exposed rats (C and D)

Table 29. DNA damage analysis in PBMCs of 9-month HBNR sand-exposed rats.

Sample code	Radiation dose (mGy)	DNA damage analysis in 9 month HBNR exposed rats using comet assay					
		Comet Length (µm)	Head Length (µm)	Tail Length (µm)	Tail Intensity (a.u)	Tail DNA%	Tail Moment (µm)
H3	69.69 ± 2.92	60.2	48.3	11.8	23.6	6.3	3.3
H6	69.69 ± 2.92	66.5	45.8	20.7	18.8	5.6	3.0
H9	69.69 ± 2.92	71.9	52.3	19.6	23.1	7.7	3.1
H12	69.69 ± 2.92	69.1	55.4	13.8	16.4	7.0	1.5
H15	69.69 ± 2.92	64.5	50.0	14.5	20.0	7.2	0.9
H18	69.69 ± 2.92	61.3	53.2	8.1	19.5	3.2	4.6
H22	69.69 ± 2.92	67.6	50.6	17.1	27.8	8.3	4.0
H25	69.69 ± 2.92	71.1	57.5	13.6	31.1	7.8	5.4
H28	69.69 ± 2.92	48.7	37.1	11.5	22.1	5.7	3.2
H30	69.69 ± 2.92	69.0	58.3	10.8	27.3	4.3	2.9
N=10	**Mean**	**65.0**	**50.8**	**14.1**	**23.0**	**6.3**	**3.2**
	SD	**6.9**	**6.2**	**4.0**	**4.6**	**1.6**	**1.3**
	Min	**48.7**	**37.1**	**8.1**	**16.4**	**3.2**	**0.9**
	Max	**71.9**	**58.3**	**20.7**	**31.1**	**8.3**	**5.4**

Table 30. DNA damage analysis in PBMCs of 9-month NBR sand-exposed rats.

Sample code	Radiation dose (mGy)	DNA damage analysis in 9 month NBR exposed rats using comet assay					
		Comet Length (µm)	Head Length (µm)	Tail Length (µm)	Tail Intensity (a.u)	Tail DNA%	Tail Moment (µm)
N1	1.79 ± 0.20	65.7	49.2	16.5	28.5	7.4	3.7
N3	1.79 ± 0.20	52.3	38.4	13.9	19.4	8.9	5.6
N5	1.79 ± 0.20	69.7	50.8	18.9	25.4	7.0	3.9
N7	1.79 ± 0.20	66.6	47.4	19.2	28.5	5.6	2.3
N9	1.79 ± 0.20	61.3	56.9	4.4	17.8	3.9	0.8
N=5	**Mean**	**63.1**	**48.5**	**14.6**	**23.9**	**6.6**	**3.3**
	SD	**6.7**	**6.7**	**6.1**	**5.0**	**1.9**	**1.8**
	Min	**52.3**	**38.4**	**4.4**	**17.8**	**3.9**	**0.8**
	Max	**69.7**	**56.9**	**19.2**	**28.5**	**8.9**	**5.6**

Table 31. DNA damage analysis in PBMCs of 18-month HBNR sand-exposed rats.

Sample code	Radiation dose (mGy)	DNA damage analysis in 18 month HBNR exposed rats using comet assay					
		Comet Length (µm)	Head Length (µm)	Tail Length (µm)	Tail Intensity (a.u)	Tail DNA%	Tail Moment (µm)
H3*							
H6	139.38±5.84	69.4	44.6	24.9	17.2	6.0	3.1
H9*							
H12	139.38±5.84	71.7	56.5	15.2	17.6	7.6	1.6
H15	139.38±5.84	67.1	47.5	19.6	19.4	7.8	1.0
H18	139.38±5.84	60.1	53.8	6.3	20.0	3.1	5.1
H22	139.38±5.84	70.4	48.7	21.7	25.9	9.1	6.3
H25	139.38±5.84	72.4	55.8	16.6	29.3	8.2	6.0
H28	139.38±5.84	44.4	35.5	8.9	23.8	6.2	3.8
H30	139.38±5.84	64.4	51.6	12.8	28.4	5.4	2.4
N=8	**Mean**	**65.0**	**49.2**	**15.7**	**22.7**	**6.7**	**3.7**
	SD	**9.3**	**6.9**	**6.3**	**4.8**	**1.9**	**2.0**
	Min	**44.4**	**35.5**	**6.3**	**17.2**	**3.1**	**1.0**
	Max	**72.4**	**56.5**	**24.9**	**29.3**	**9.1**	**6.3**
*Rats died during experiment due to unknown reason; not able to obtain data.							

Table 32. DNA damage analysis in PBMCs of 9-month NBR sand-exposed rats.

Sample code	Radiation dose (mGy)	DNA damage analysis in 18 month NBR exposed rats using comet assay					
		Comet Length (µm)	Head Length (µm)	Tail Length (µm)	Tail Intensity (a.u)	Tail DNA%	Tail Moment (µm)
N1	3.58±0.40	63.8	49.5	14.3	27.4	7.1	3.9
N3	3.58±0.40	52.3	37.4	14.9	19.4	8.7	5.7
N5	3.58±0.40	71.8	51.9	19.9	25.3	7.0	3.9
N7	3.58±0.40	66.9	47.4	19.5	28.5	4.2	2.3
N9	3.58±0.40	61.4	51.9	9.5	21.1	4.4	1.2
N=5	**Mean**	**63.2**	**47.6**	**15.6**	**24.3**	**6.3**	**3.4**
	SD	**7.2**	**6.0**	**4.3**	**4.0**	**1.9**	**1.7**
	Min	**52.3**	**37.4**	**9.5**	**19.4**	**4.2**	**1.2**
	Max	**71.8**	**51.9**	**19.9**	**28.5**	**8.7**	**5.7**

Table 33. Influence of background radiation on DNA damage in PBMCs of rats exposed to HBNR and NBR sand

Exposure duration (Months)	Type of exposure	Radiation dose (mGy)	N	DNA damage analysis using comet assay [Mean±SD (Min–Max)]						
				Comet Length (µm)	Head Length (µm)	Tail Length (µm)	Tail Intensity (a.u)	Tail DNA%	Tail Moment (µm)	P-value
9	HBNR	69.69 ± 2.92	10	65.0±6.9 (48.7–71.9)	50.8±6.2 (37.1–58.3)	14.1±4.0 (8.1–20.7)	23.0±4.6 (16.4–31.1)	6.3±1.6 (3.2–8.3)	3.2±1.3 (0.9–5.4)	**0.933**
	NBR	1.79±0.20	5	63.1±6.7 (52.3–69.7)	48.5±6.7 (38.4–56.9)	14.6±6.1 (4.4–19.2)	23.9±5.0 (17.8–28.5)	6.6±1.9 (3.9–8.9)	3.3±1.8 (0.8–5.6)	
18	HBNR	139.38±5.84	8	65.0±9.3 (44.4–72.4)	49.2±6.9 (35.5–56.5)	15.7±6.3 (6.3–24.9)	22.7±4.8 (17.2–29.3)	6.7±1.9 (3.1–9.1)	3.7±2.0 (1.0–6.3)	**0.813**
	NBR	3.58±0.40	5	63.2±7.2 (52.3–71.8)	47.6±6.0 (37.4–51.9)	15.6±4.3 (9.5–19.9)	24.3±4.0 (19.4–28.5)	6.3±1.9 (4.2–8.7)	3.4±1.7 (1.2–5.7)	

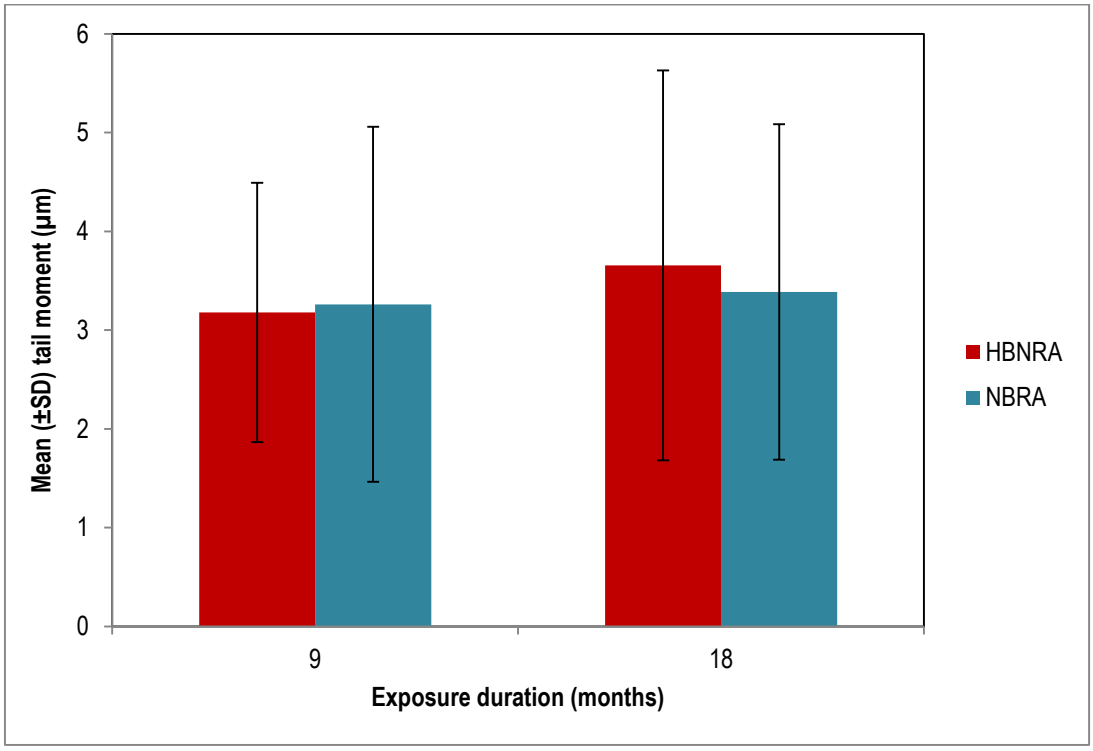


Fig. 17. Influence of background radiation on DNA damage (tail moment) in rat PBMCs

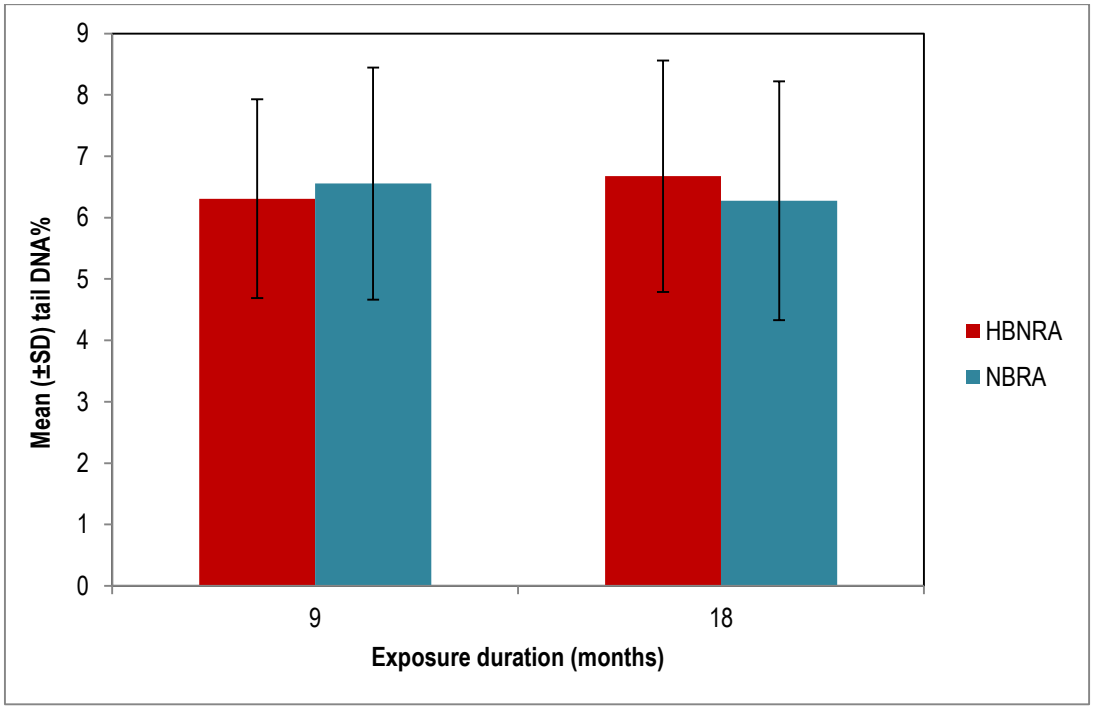


Fig. 18. Influence of background radiation on DNA damage (tail DNA%) in rat PBMCs

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SUMMARY

Everyone is exposed to natural radiation. The main sources include cosmic rays and naturally occurring terrestrial radionuclides present in the earth's crust, in building materials and in air, water and foods and in the human body itself. More than 60 radionuclides can be found in the environment, which can be divided into three general categories: primordial, cosmogenic, and manmade. A significant amount of natural radiation is due to radon gas (^{222}Rn), which is released from the soil and may concentrate in dwellings. The accumulation of large amount of radionuclides in soil and rocks, such as ^{232}Th, ^{238}U, ^{226}Ra, ^{40}K, etc., in some parts of the world (Ramsar in Iran, Yangjiang in China, Kerala in India, Guarapari in Brazil, etc.) release considerably high level of radiation.

Stochastic effects are usually seen in inhabitants who receive background radiation exposure above a certain level. The worldwide annual per capita effective annual dose (mSv) of natural background radiation is 2.4 mSv, varying over an order of magnitude depending upon site-specific natural radioactivity reaching up to 10-20 mSv. These areas or a complex of dwellings where the sum of cosmic radiation and natural radioactivity in soil, indoor and outdoor air, water, food, etc. leads to chronic exposure situations from external and internal exposures that result in an annual effective do*se* to the public above a defined level is known as high background natural radiation areas

This study was designed with the following objective:

- To analyze the frequency of MN, CAs, DNA damage in blood samples of HBNR and NBR sand-acclimatized rats using cytogenetic techniques.

Wistar rats were exposed to natural background radiation, at the radiation dose of 92.91±3.89 mGy y^{-1} for a period of 24 months and a parallel control set exposed to dose of 2.38 ± 0.26 mGy y^{-1} was also maintained. Blood samples were collected from the rats exposed to sand collected from a high background natural radiation (HBNR) area and from rats exposed to sand collected from normal background radiation (NBR) area and were subjected to cytogenetic analysis.

Several cytogenetic techniques such as cytokinesis-block micronucleus (CBMN) assay, chromosomal aberrations (CA) and comet assay was carried out in Wistar rats exposed to HBNR and NBR sand bed. The distributions and frequencies of MN in peripheral blood lymphocytes of rats exposed to HBNR and NBR sand at interval of 3 months up to 18 months were analyzed. No statistical difference in MN frequency between HBNR and NBR exposed rats was seen in any of the time intervals. This may be due to the level of radiation exposure is too low to induce significant MN. On the other hand, it is possible that damage is induced, but repaired, in rats exposed to high background radiation sand. However, an age-dependent increase in the frequency of MN was observed. Age-dependent increase was most likely due to an increase in acentric fragments produced by unrepaired DNA strand-breaks induced either by exposure to environmental pollutants or endogenously or by spindle disturbances resulting in chromosome lagging. It has been reported in studies that, in old age, biomarkers of genomic instability, such as MN, are more common in the peripheral lymphocytes.

Chromosomal aberration studies were carried out in Wistar rats. The blood drawn at interval of 6 months, maximum up to 18 months was analyzed for aberrations such as chromosomal gaps, breaks, dicentrics, rings and fragments. No

statistical difference was found in CAs between HBNR and NBR exposed rats. This may be due to the level of radiation exposure is too low to induce significant CAs. However, an age-dependent increase in the frequency of CAs was observed in rats exposed to HBNR and NBR sand bed.

The DNA damage of the cell was primarily assessed by Tail DNA%, Tail length and Tail moment using comet assay technique. The samples obtained at 9- and 18-month-exposed rats were used for DNA damage analysis. No significant DNA damage was found in rat peripheral blood mononuclear cells (PBMCs) from HBNRA sand bed compared to DNA damage from the NBRA sand bed. Studies from HBNRA of Kerala did not show significant difference in basal DNA damage in individuals compared to individuals from the NBRA. But studies from Ramsar, Iran reported significantly elevated spontaneous DNA damage in subjects. The discrepancy in the results seen in this study and from Ramsar could be due in part to the differences in the exposure conditions.

ACHIEVEMENTS OF THE PROJECT

From the findings of the Project it was comprehensively proved that chronic exposure to high natural background radiation did not have any significant impact on the chromosomal setup (micronucleus number, chromosomal aberrations and DNA damage) of rats.

CONTRIBUTION TO THE SOCIETY

There is a general belief among the public that inhabitants of high natural background radiation areas, i.e. those receiving high background radiation exposure chronically may be have higher levels of micronuclei, chromosomal aberrations and DNA damage. This study was carried out under laboratory conditions in Wistar rats to verify such claims. The results of the study imply that the abovesaid micronucleus number, chromosomal aberrations and DNA damage in high background radiation-exposed rats are not different from those of non-exposed ones. This may be due to the level of radiation exposure is too low to induce significant micronuclei, chromosomal aberrations and DNA damage. It is also possible that damage is induced, but repaired, in rats exposed to high background radiation sand. However, an age-dependent increase in the frequency of micronuclei, chromosomal aberrations and DNA damage was observed. However, such values were similar in both the experimental and control rats.

Consolidated Statement of Expenditure incurred on the Major Research Project

(for the complete Project Period)

Name of the Project Investigator & Department : Dr. S. Godwin Wesley & Zoology

Title of the Project :

"Study of the Effect of Natural Background Radiation on Certain Rodents (in Vitro) – A Cytogenetic Approach"

File No. 43-555/2014 (SR), dt. December 2015

A. Non-Recurring

1. **Books & Journals:**

Sl. No.	Name of the Books Purchased	Bill Nos.	Date	Amount Rs.
1.	Cytogenetic assays of environmental mutagens	407-6793807-2581946	24.03.2017	1722.72
2.	Molecular Cytogenetics: Protocols and Applications	407-6793807-2581946	24.03.2017	2530.83
3.	Cytogenetics: Techniques and Applications	407-0363771-9179554	24.03.2017	5557.00
4.	The Rat: Reference Tables and Data for the Albino Rat and the Norway Rat	407-6863582-4379535	25.03.2017	1259.00
	Total			11069.55
	Round off			11070.00

2. Equipment

Sl. No.	Name of the Firm	Name of the Equipment Purchased	Bill No.	Date	Amount
1	M/s. The Empire Scientific Company 60, South Car Street, Nagercoil – 629 001	Research Microscope (Trinocular Version) – CX21i	R329	16.08.2016	265779
2	M/s. Ponmani & Co. New No. 140, Old No. 73, Avarampalayam Road, New Siddhapudur, Coimbatore – 641044	Cytogenetics Kit	57718	22.11.2016	18187
3	M/s. Ponmani & Co. New No. 140, Old No. 73, Avarampalayam Road, New Siddhapudur, Coimbatore – 641044	Streptomycin Standard Solution	57765	24.11.2016	4646
4	Allied Scientific Products 39/19B Gopal Nagar Road, Kolkata – 700027	96 Well Comet Assay Kit	753	24.10.2017	33899
5	M/s. Premier Chemicals & Instruments Co. 181/1, St. Michael Building, Mathias Nagar, K.P. Road, Nagercoil – 1	Stereo Zoom Trinocular head inclined at 45 degree interpapillary 52-75 mm Aluminum die	544	18.10.2018	77486
				Total	399997

B. Recurring

1. Honorarium to Retd. Teacher @ Rs. 18000/- p.m.

Sl. No.	Month	Amount
1.	April 2016	18000
2.	May 2016	18000
3.	June 2016	18000
4.	July 2016	18000
5.	August 2016	18000
6.	September 2016	18000
7.	October 2016	18000
8.	November 2016	18000
9.	December 2016	18000
10.	January 2017	18000
11.	February 2017	18000
12.	March 2017	18000
13.	April 2017	18000
14.	May 2017	18000
15.	June 2017	18000
16.	July 2017	18000
17.	August 2017	18000
18.	September 2017	18000
19.	October 2017	18000
20.	November 2018	18000
21.	December 2018	18000
22.	January 2018	18000
23.	February 2018	18000
24.	March 2018	18000
25.	April 2018	18000
26.	May 2018	18000
27.	June 2018	18000
28.	July 2018	18000
29.	August 2018	18000
30.	September 2018	18000
31.	October 2018	18000
32.	November 2018	18000
33.	December 2018	18000
34.	January 2019	18000
35.	February 2019	18000
36.	March 2019	18000
	Total	648000

2. Contingency

Sl. No.	Name of the Item Purchased	Amount	Bill No. / Date
1.	Stationary	849	33/2016-17
2.	Door Coleset	1500	224/23.02.2017
3.	Chemicals	10000	22.07.2017
4.	Class wire	18048	22.07.2017
5.	Stationary	6039	13.09.2017
6.	Chemicals	6126	36/11.12.2017
7.	Chemicals	14321	09.01.2018
8.	Audit Fee	2500	18.03.2018
9.	Audit Fee	2750	9/09.07.2018
10.	Chemicals	29867	03.07.2018
11.	Chemicals	11342	06.11.2018
	Total	103342	

3. Hiring Service

Sl. No.	Description	Amount
1.	Sub-staff Allowance (2500 x 22)	55000
2.	Internet browsing	6500
3.	Typing work	8000
4.	Book Binding & Xerox	5750
		75250

4. Travel & Field Work

Sl. No.	Place Visited	Mode of Travel	Date	Amount	
1.	Trivandrum	Taxi	16.08.2016	4500	The P.I. has visited the following places with regard to Data Collection to carry out the project only
2.	Quilam	Taxi	27.08.2016	5600	
3.	Tirunelveli	Taxi	22.09.2016	3400	
4.	Malavalakurich	Taxi	13.10.2016	2700	
5.	Trivandrum	Taxi	02.11.2016	4700	
6.	Colachel	Taxi	18.11.2016	1800	
7.	Muttam	Taxi	07.12.2016	2400	
8.	Quilam	Taxi	21.12.2016	5250	
9.	Trivandrum	Taxi	13.01.2017	4500	
10.	Kanyakumari	Taxi	21.01.2017	1300	
11.	Thenkaipattanam	Taxi	08.02.2017	2000	
12.	Tuticorin	Taxi	17.03.2017	6700	
13.	Muttam	Taxi	17.04.2017	2500	
14.	Manavalakurich	Taxi	23.05.2017	2700	
15.	Quilam	Taxi	12.06.2017	5600	
16.	Trivandrum	Taxi	26.07.2017	4700	
17.	Rajakkamangalam	Taxi	03.02.2018	2600	
18.	Colachel	Taxi	28.04.2018	1800	
19.	Poovar	Taxi	18.06.2018	3000	
20.	Tuticorin	Taxi	08.08.2018	6300	
21.	Thenkaipattanam	Taxi	04.10.2018	2000	
		Total		**76050**	

5. Over Head

Sl. No.	Description	Amount
1.	Over Head	79800
	Total	79800

ABSTRACT

Sl. No.	Head	Amount
A. Non-Recurring		
1.	Books & Journals	11070
2.	Equipments	399997
B. Recurring		
3.	Honorarium	648000
4.	Contingency	103342
5.	Hiring Service	75250
6.	Travel & Field Work	**76050**
7.	Over Head	79800
	Total	**1393509**

23.01.2023

Scott Christian College (Autonomous) Principal Investigator Principal

University Grants Commission
Bahadurshah Zafar Marg
New Delhi – 110 002

ACCESSION CERTIFICATE

This is certify that Dr. S. Godwin Wesley, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil has handed over the following books and journals purchased under the scheme of Major Research Project to the Library of Scott Christian College (Autonomous), Nagercoil.

Sl. No.	Name of the Books Purchased
1.	Cytogenetic assays of environmental mutagens
2.	Molecular Cytogenetics: Protocols and Applications
3.	Cytogenetics: Techniques and Applications
4.	The Rat: Reference Tables and Data for the Albino Rat and the Norway Rat

Signature of the Librarian

Signature of the Principal Investigator

Signature of the Principal

University Grants Commission
Bahadurshah Zafar Marg
New Delhi – 110 002

ASSETS CERTIFICATE

This is certify that Dr. S. Godwin Wesley, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil has handed over the following equipment purchased under the scheme of Major Research Project to the Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil.

Sl. No.	Name of the Equipment
1	Research Microscope (Trinocular Version) – CX21i
2	Cytogenetics Kit
3	Streptomycin Standard Solution
4	96 Well Comet Assay Kit
5	Stereo Zoom Trinocular head inclined at 45 degree interpapillary 52-75 mm Aluminum die

Signature of the
Head of the Department

Signature of the
Principal Investigator

Signature of the
Principal

15.05.2018

RECEIPT

Received Rs. 79,800/- (Rupees Seventy nine thousand and eight hundred only) from the University of Grants Commission vide its letter 43-555/2014 (SR), dt. December 2015 for the Major Project "Study of the Effect of Natural Background Radiation on Certain Rodents (in Vitro) – A Cytogenetic Approach" to Dr. S. Godwin Wesley, Department of Zoology as institutional charges

Principal