IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 7, Issue 4 (Jul. – Aug. 2021), PP 26-33
www.iosrjournals.org

A study of comparison between effect of gamma rays and X rays (1.2 Gy) on chromosomes of common house musk shrew, *Suncus murinus.*

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Abstract
Physical mutagens as X rays, γ rays induce fatality on living system as chromosomal aberrations that eventually lead to cancer. Chromosomal Aberration Test (CAT) is employed as an index of cancer. In the present study, the author has investigated the effect of two different radiations i.e. X rays and γ-rays (1.2 Gy dose) on bone marrow cells of house musk shrew, Suncus murinus. The experimental animals were exposed to whole body irradiations in two different sets. Seven different types of chromosomal aberrations were observed in bone marrow cells of the shrew, after four different time intervals post exposure, as 1 hr, 16 hr, 48 hr and 1 week (168 hr.) As a result, for both the exposures, the maximum percentage of aberration had been obtained after 48 hr of exposure for both the radiations while minimum was observed after 1 week of exposure. On the other hand, when compared between types of aberrations, the maximum frequencies of aberration in mitotic chromosomes were observed with centromeric dissociation for both the rays, while ring chromosome scored the minimum. Further, statistical analysis of the data reveals that the aberrations are non-random in distribution, they are somewhat time-dependent and centromeric regions of some chromosomes are most vulnerable to the radiations.

Key-words: γ-ray, chromosomal aberrations, shrew, Suncus murinus.

Date of Submission: 20-06-2021 Date of Acceptance: 05-07-2021

I. Introduction

Chromosomal aberrations lead to cancer. In this view, such aberrations had been studied in wide varieties of animals as mice, fish, grasshoppers, monkeys, and even plants etc. (Muller (1927) on artificial mutagenesis in *Drosophila*, Krause and Ziegler (1906) on mammalian tissue, Stadler (1928) on maize chromosome, Goodspeed and Olson (1928) on *Nicotiana* sp, Amato (1911) ; Grasnick (1918) on amphibian eggs and larva; work on common Rhesus monkey, *Macaca mulatta* as Koshichenko and Semenov (1975), Van and Paul (1976) etc.). Other notable workers in this field are Mohr (1919); Carlson (1938, 1940, 1941a, 1941b, 1949); Mazumder and Manna (1967); Manna and Mazumder (1962, 1967, 1968); Holleander (1964); Sur and Manna (1987); Sur (1998); Sur and Pandey (2004). Sarkar and Manna (2001) studied the effect of nitrogen fixing bacteria *Beijerinckia indica* on three mammalian models i.e. shrew, monkey and cat.

We have been studying chromosomal aberrations in laboratory models as grasshoppers, fish, mice and shrew for last few years (Sur, *et al,* 2010, 2011, 2012(a), 2012(b), 2012(c); Das *et al,* 2011, 2013). Even, radio-protective activities of leaf extracts from *Barleria lupulina* in mice and fish had been reported by us (Sur and Das (2012(a), 2012(b)).

To study and compare the effect between X rays and γ rays in wild animal, the present study had been undertaken on shrew.

II. Materials And Methods

Collection of animals and maintenance:

Adult healthy house musk shrews (*Suncus murinus*) of both sexes were collected from their natural habitat as from hedges, near drains, human dwellings, under concrete slabs, etc., during nights, when the animals come out of their dwellings for search of food. The animals were transferred to laboratory and acclimatized for a short period of time, with food and water. The study is cleared by Animal Ethical Committee, Dept of Zoology, Kalyani University, West Bengal, India.

Radiation:

After acclimatization, the animals were subjected to whole body γ irradiation from $_{27}Co^{60}$ γ-ray source and X rays from an X ray apparatus with Al as filter (dose = 1.2 Gy). The shrew were caged in special container and subjected to the radiations in sets of three shrews for each hour (Table 1).

After exposure, the shrews were transferred to the laboratory. They were chloroformed and sacrificed after four different time intervals i.e. 1 hr, 16 hr, 48 hr and 1 week (the shrew didn't live after 1 wk of exposure). Mitotic chromosomes of the animals were studied from well spread metaphase plates prepared from femur bone marrow cells of the hind legs of dissected animals (Fig 1). The bone marrow was suspended in 1% sodium citrate solution (hypotonic solution) and was flushed to bring the cells into a uniform suspension. Then cells were fixed in aceto-alcohol (acetic acid: alcohol – 1:3 v/v). Slides were prepared by dropping the cells on chilled slides maintained at -5°C. Staining was done by flooding the slides with Giemsa staining solution for one and half hour. Data was scored by using 300 cells for each time intervals. Analysis of data was done by using different types of standard statistical tools as per Snedecor G.W. & Cochran W.G. (1967). A total of 48 shrews were taken as the test series for both the doses, while the same number of individuals served as control. (Table 1).

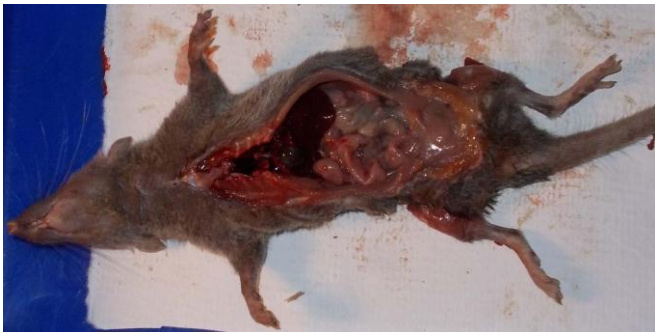


Fig 1: Dissected House musk shrews (*Suncus murinus*)

Table 1: Number of shrews (male and female) irradiated with γ-ray & X ray (dose= 1.2 Gy) and their bone-marrow cells fixed at different time intervals**:**

Fixation time	γ-ray 1.2 Gy dose				X-ray 1.2 Gy dose			
	Number of males	Number of females	Number in total	Number died	Number of males	Number of females	Number in total	Number died
1 hr	3	3	6	-	3	3	6	-
16 hr	3	3	6	2	3	3	6	-
48 hr	3	3	6	3	3	3	6	1
1 week (168 hr.)	3	3	6	-	3	3	6	-

III. Results

a. For 1.2 Gy γ ray dose

Control Series:

Keeping in accordance with the Treated Series, a total of 300 mitotic cells for each hour, summing up to 1200 cells in 4 different time-intervals each were studied in the control series. Therefore, all total 48,000 mitotic chromosomes were studied in this whole control series. No significant aberrations were found in this control series.

Treated Series:

Similarly, in this series, a total of 1200 cells and therefore 48,000 chromosomes were studied. Examination of chromosomes after γ-irradiation showed that the rays were capable of inducing seven different types of chromosomal aberrations, as chromatid break, iso chromatid gap, iso chromatid break, sub chromatid gap , centromeric dissociation, translocation and ring chromosome Out of these different types of aberrations, maximum frequency of aberration was obtained with centromeric dissociation (2.04%) while minimum was obtained with ring chromosome (0.47%) (Table 2, Fig. 2, Fig. 5).

When compared with respect to time, it was found out that chromosomal aberration increased slowly from 1hr (6.08%) to 48 hr post exposure to γ-ray, was maximum at 48th hr (10.25%), and then decreases and becomes negligible after 1 week of exposure (4.87%) (Table 2, Fig. 3).

Statistical analysis of the data reveals that the χ^2 (Chi square) value for the treated series are 9.96** for Chromatid break, 25.02** for Iso chromatid break, 11.29** for sub chromatid gap, 15.40** for centromeric

dissociation, 12.42** for translocation, 9.54** ring chromosome and 59.22** for the pooled data. These values are highly significant. The overall F-value = 81.90** which is highly significant (Table 4).

b. For 1.2 Gy X ray dose

Control Series:

In this series, similarly 1200 bone marrow cells and therefore 48,000 mitotic chromosomes were studied. Negligible chromosomal aberrations had been observed herein also.

Treated Series:

Similarly, in this series, seven types of structural chromosomal aberrations were observed also; the maximum had been scored with centromeric dissociation (1.05%) and minimum with ring chromosome (0.18%) (Table 2, Fig. 2, Fig. 4).

In terms of time intervals, the highest aberration was obtained after 48 hr. (7.12%) and lowest was after 1 week (4.68%) post exposure (Table 2, Fig. 3).

The χ^2 value for this treated series are 17.91** for chromatid break, 33.21** for Iso chromatid gap, 11.87** for Iso chromatid break, 12.44** for sub chromatid gap, 14.21** for Centromeric dissociation. The overall the χ^2 value is 18.53**for the pooled data. All these values are highly significant. The overall F-value = 36.72** (Table 3).

Table 2: X-ray and γ-ray (1.2 Gy) irradiated chromosomal aberrations in treated shrews

Time	rays	NC	No. of chromosomes	Types of Aberrations							Total aberrations	Total aberration percentage
				chromatid break	iso chromatid gap	iso chromatid break	sub chromatid gap	centromeric dissociation	translocation	ring chromosome		
1 hr.	X ray	300	12000	102	57	36	78	208	68	58	607	5.06
	γ ray	300	12000	115	92	59	65	245	85	68	729	6.08
16 hr.	X ray	300	12000	96	57	88	153	254	37	22	707	5.89
	γ ray	300	12000	143	103	75	74	218	75	44	732	6.1
48 hr.	X ray	300	12000	155	61	103	159	244	77	55	854	**7.12**
	γ ray	300	12000	176	168	157	168	319	166	76	1230	**10.25**
1 wk.	X ray	300	12000	172	18	88	57	177	33	17	562	**4.68**
	γ ray	300	12000	112	75	42	54	196	66	39	584	**4.87**
Total abrr	X ray	1200	48,000	525	193	315	447	883	215	152	2730	3.25
	γ ray	1200	48,000	546	438	333	361	978	392	227	3275	6.82
Total % Aberrations			X ray	0.63	0.23	0.38	0.53	1.05	0.26	0.18		
			γ ray	1.14	0.91	0.69	0.75	2.04	0.82	0.47		

C= Control Series, T= Treated Series, NC= Number of cells, wk= week, abrr = aberrations

Table 3: Statistical analysis of effect of X-rays (1.2 Gy) on mitotic chromosomes in shrew

Aberration Statistics	Chromatid break	Iso chromatid gap	Iso chromatid break	Sub chromatid gap	Centromeric dissociation	Translocation	Ring chromosome	Total aberration
Control S.E. +	2.33	2.29	1.29	1.71	3.12	0.91	1.55	5.58
Treatment S.E.+	18.78	10.13	14.68	25.93	17.61	11.01	10.75	64.7
Control C.D. at 5%	4.56	4.48	2.53	3.35	6.11	1.79	3.05	10.94
Treatment C.D. at 5%	37.2	19.85	28.78	50.83	34.52	21.56	21.06	120.81
Control C.D. at 1%	5.99	5.89	3.32	4.4	8.03	2.35	4	14.38
Treatment C.D. at 1%	48.87	26.08	37.81	66.78	45.35	28.35	27.67	166.6
t - Values	6.47**	3.90**	4.26**	4.09**	11.74**	3.96**	3.18**	9.57**
Chi-Square Values	17.91**	33.21**	11.87**	12.44**	14.21**	1.89	3.81	18.53**
r - Values	-1	-0.89	-0.43	-0.25	-0.2	0.99**	0.62**	-0.73

Over all F value = 36.72 **

* Significant at 5% level

** Significant at 1% level

Standard analysis of the data were undertaken as Standard Error (S.E) ; Critical Difference (C.D) at 1% and 5 % level; t- test ; chi- square analysis and correlation coefficient (r-value) and Analysis of Variance (F-Value) of both Treatment and Control species.

Table 4: Statistical analysis of effect of γ-rays (1.2 Gy) on mitotic chromosomes in shrew

Aberration Statistics	Chromatid break	Iso chromatid gap	Iso chromatid break	Sub chromatid gap	Centromeric dissociation	Translocation	Ring chromosome	Total aberration
Control S.E. +	2.74	1.25	1.58	1.29	4.73	1.22	1.49	7.91
Treatment S.E.+	14.9	20.33	25.49	26.24	26.78	23	9.01	141.37
Control C.D. at 5%	5.37	2.45	3.1	2.53	9.28	2.40	2.93	15.5
Treatment C.D. at 5%	29.21	39.85	49.96	51.43	52.49	45.07	17.66	277.08
Control C.D. at 1%	7.05	3.22	4.07	3.32	12.19	3.15	3.84	20.07
Treatment C.D. at 1%	38.37	52.36	65.64	67.56	68.95	59.22	23.21	364.02
t - Values	7.42**	4.95**	2.44*	3.21	8.17**	3.60**	5.58**	5.06**
Chi-Square Values	9.96**	7.09*	25.02**	11.29**	15.40**	12.42**	9.54**	59.22**
r - Values	-0.79	-0.63	-0.72	-0.31	-0.11	-0.81	-0.74	-0.99

Over all F value = 81.90**

* Significant at 5% level

** Significant at 1% level

Standard analysis of the data were undertaken as Standard Error (S.E) ; Critical Difference (C.D) at 1% and 5 % level; t- test ; chi- square analysis and correlation coefficient (r-value) and Analysis of Variance (F-Value) of both Treatment and Control species.

Fig. 2: Bar-Diagram comparing seven different types of chromosomal aberrations in bone marrow cells of shrew induced by X rays and γ-rays (1.2 Gy)

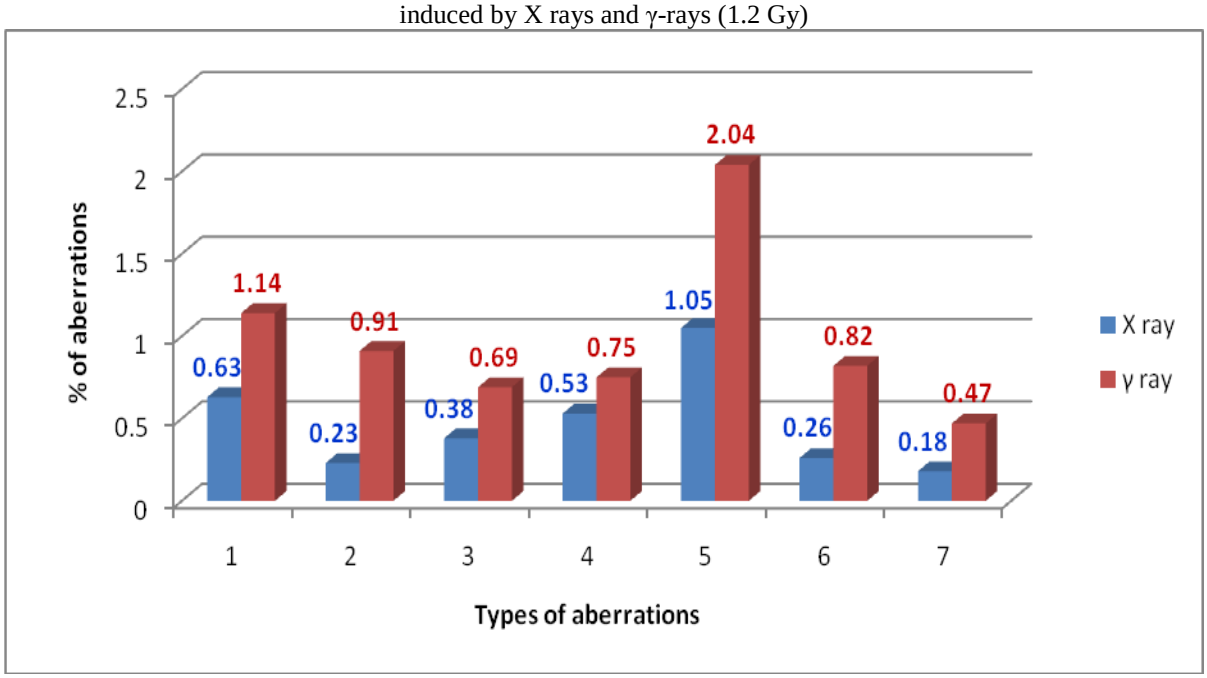
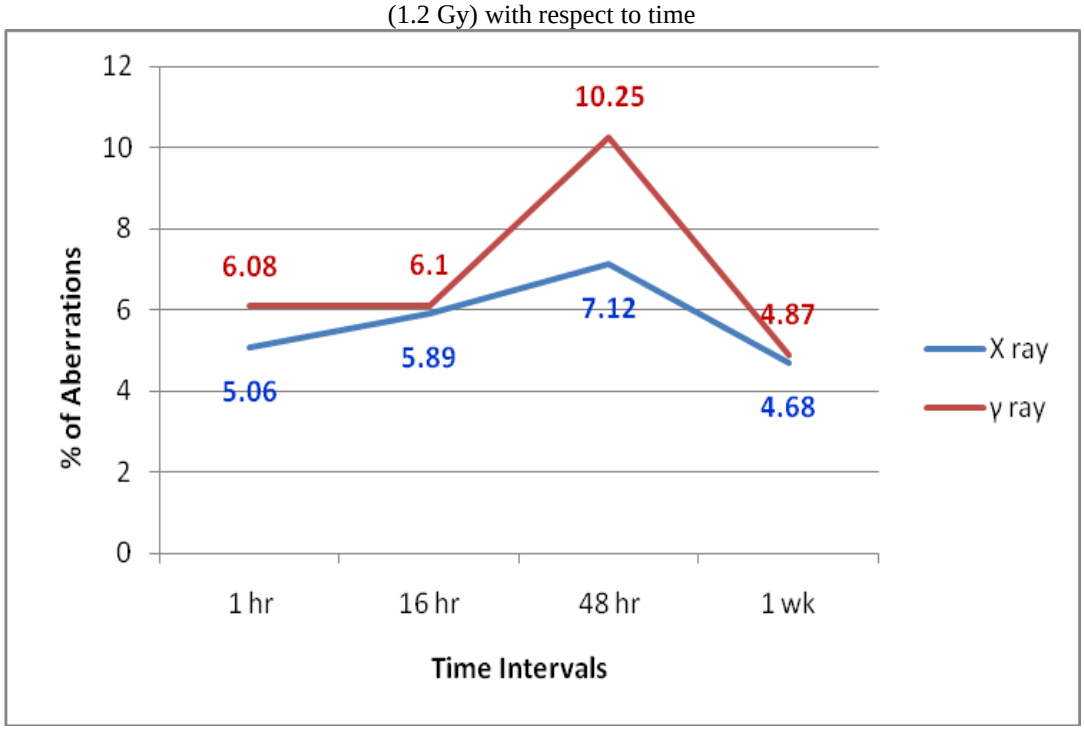


Fig. 3: Line-Diagram showing chromosomal aberrations of shrew induced by X rays & γ-ray (1.2 Gy) with respect to time



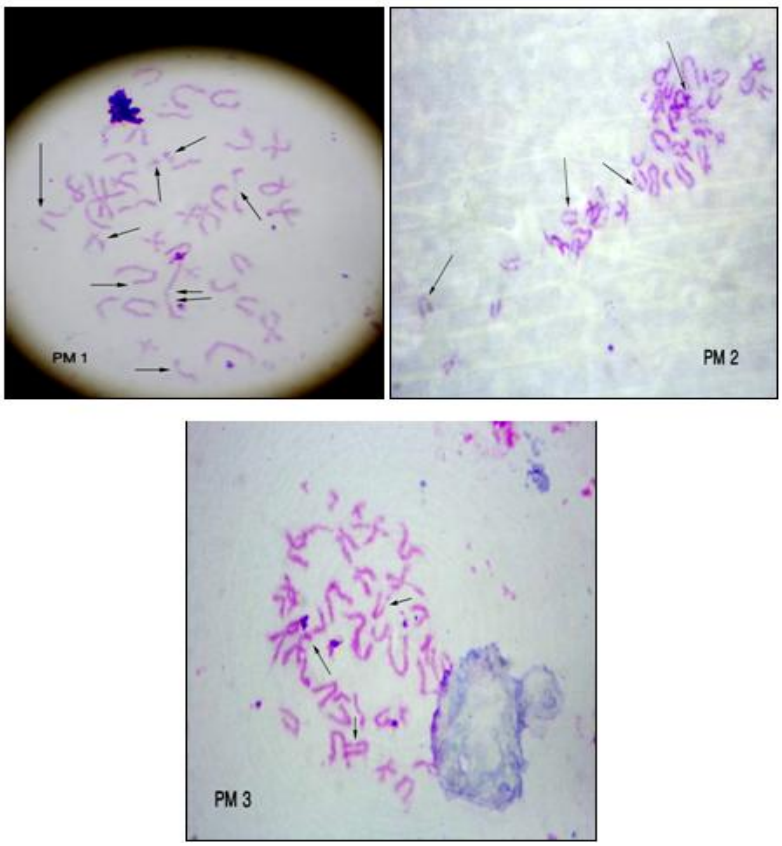


Fig 4: Photomicrographs showing various chromosomal aberrations of shrew treated by X ray (1.2 Gy)

PM 1: iso chromatid gap, chromatid break, sub chromatid gap, centromeric dissociation,
PM 2: translocation, sub chromatid gap, iso chromatid break
PM 3: translocation, sub chromatid gap

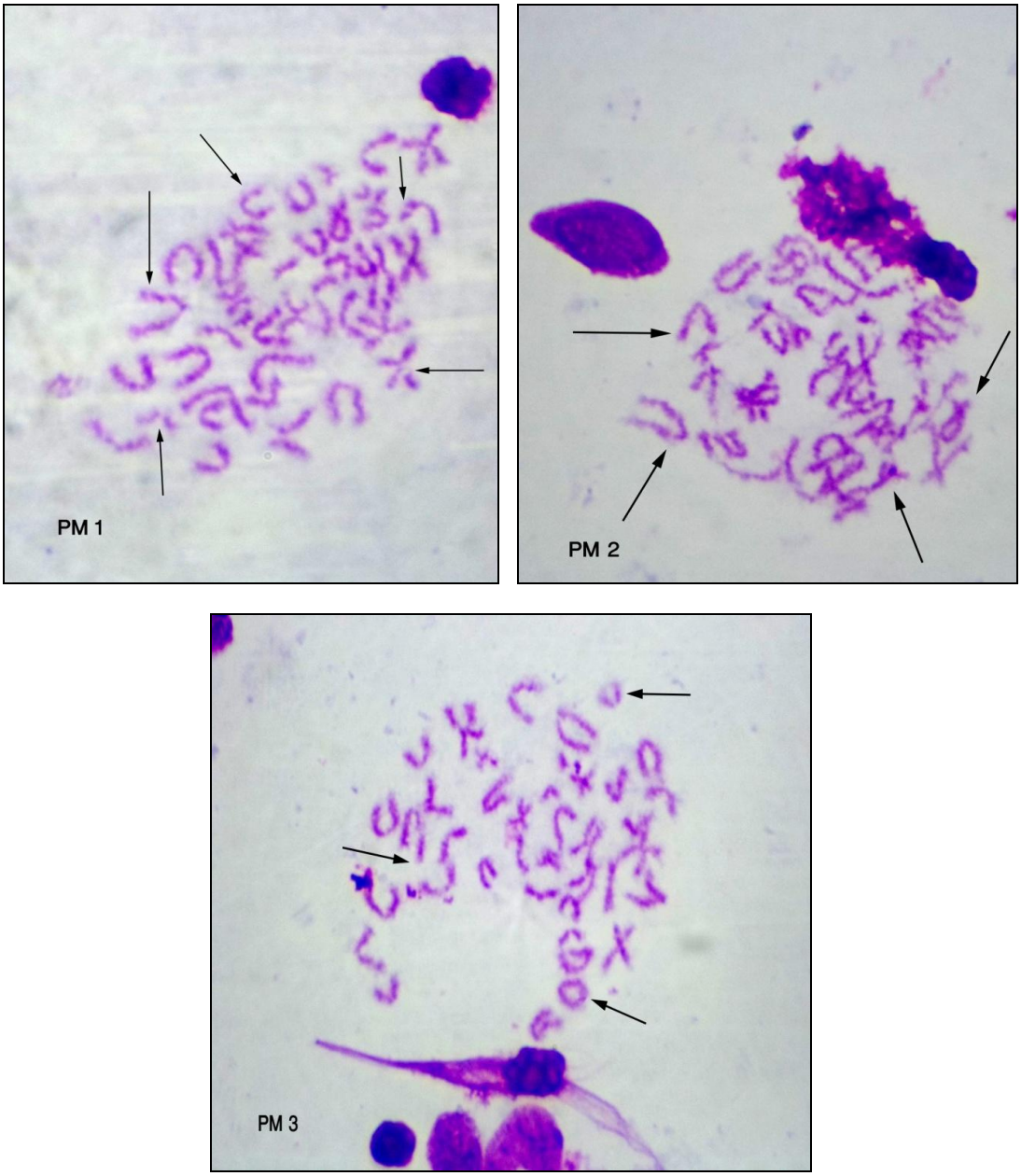


Fig 5: Photomicrographs (PM) showing various types of chromosomal aberrations in bone marrow cells of shrew treated with 1.2 Gy γ-ray

PM 1: chromatid break, centromeric dissociation, sub chromatid gap, iso chromatid gap
PM 2: iso chromatid break, translocation.
PM 3: chromatid break, centromeric dissociation, ring chromosome.

IV. Discussion

Suncus murinus belongs to the family Soricidae (Ellerman JR and Morrison-Scott TCS, 1951). First report on somatic chromosome number of *S murinus* seems to have been done in 1938 sited by Borgaonkar. The diploid number 40 was subsequently confirmed by Manna and Talukdar (1967) and Ray-Chaudhuri *et al.* (1968) for the Indian taxon. The chromosome map of *Suncus* was not available as such till 2001 as reported by Kuroiwa, A. et al. (2001). The reserachers used direct R-banding fluorescence in situ hybridization (FISH) method to map 18S-28S ribosomal RNA genes and 10 human cDNA clones on the chromosomes of the musk shrew (*Suncus murinus*). The comparative mapping of 10 cDNA clones of human chromosome 1 demonstrated that human chromosome 1 consisted of at least three segments homologous to *Suncus* chromosomes (chromosomes 7, 10, and 14).

Earlier, Sur, Das and Sarkar (2012); Sur, Das and Das (2012); assessed the effect of two different doses of X-rays (80r and 120r) on bone marrow cells of mice, *Mus musculus.* Moreover; Das, Sur and Das (2011) and Sur *et al.,* (2012(c)) further investigated the effect of two different doses of γ-rays (80r and 240r) on bone marrow cells of mice and shrew respectively. The present study was undertaken to investigate and compare the effect of one dose of X rays and γ-rays (1.2 Gy) on bone marrow cells of wild mammal, *Suncus murinus.* Earlier it was observed that the dose 240r γ-rays is more genotoxic to mice and shrew chromosomes than 80r, the aberrations are non-random in distribution, they are somewhat time-dependent and centromeric regions of the chromosomes are most vulnerable to γ-irradiation. Same observations are reported in the present study. But 240r dose of γ-rays was also observed to induce sub-lethal physiological changes as accumulation of peritoneal fluid in stomach and tumor formation near the nose region in the mice. But such events didn't occur in the shrews of present study.

Acknowledgements

We extend our sincere thanks to Prof. Indranil Manna (Ex-Director) & Lt. Dr. Debabrata Basu and Mr. Biswanath Kundu, Bioceramics & Coating Division, Central Glass & Ceramic Research Institute, 196 Raja S C Mullick Road, Kolkata-700032 (INDIA), for providing the necessary gamma radiation facility.

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Pranab Kumar Das. "A study of comparison between effect of gamma rays and X rays (1.2 Gy) on chromosomes of common house musk shrew, Suncus murinus.." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 7(4), (2021): pp. 26-33.