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Gamma radiations induced aberrations in bone marrow chromosomes of Swiss albino mice

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ABSTRACT

Bone marrow chromosomes are known to be highly radiosensitive. The current study seeks to examine the changes in chromosomal morphology post exposure to gamma radiation in Swiss albino mice. Observation of slides of control mice show no significant damage in chromosomes number (40) and morphology. However, after administration 0.20 Gy, 0.40 Gy, 0.60 Gy, 0.80 Gy of 60 CO-gamma rays a number of abnormalities were observed. Chromosome fragments, breaks, appearance of rings, dicentric chromosomes were found in all cases. The only difference was in their frequency. When the doses were higher the variations were observed more frequently. However, at a dose of 0.60Gy and 0.80 Gy in addition to the aforesaid abnormalities, aneuploidy was also observed. Bone marrow cells showing such defective morphology possibly may also suffer from attenuation of their genetic, physiological and biochemical mechanism(s). These observations indicate the sensitivity of the genomic apparatus of mice subjected to low doses of gamma radiations. The biomedical importance of this study can be easily visualized in the possible cytogenetic effects that would influence the generations to come. The rampant use of this radiation therefore warrants further, indepth investigation in view of the long term genetic hazards and impairment of fertility of an individual due to gamma rays.

Key words: Aneuploidy · chromosome morphology · ionized radiation · mitotic index

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INTRODUCTION

Natural background radiation of various forms exists in the biosphere and comes from three well known and studied sources *i.e.*, cosmic rays, living cells and earth crust. Living cells, which have the inherent capability to bio-accumulate and bio-amplify radioactive isotopes from the environment. A variety of radioactive elements such as radium, thorium and uranium are present in the earth's crust and emit α, β, γ-rays. Such radioactive elements are extracted and put to use in various industries, nuclear weapons test explosions, medicine, power generation, agriculture and radio-sterilization (Singh, 2011; Waghmareet al. 2013; Zalewska et al. 2014). In addition to the aforesaid useful effects certain radiations are also the principle causative factors for somatic lesion, necrosis; carcinogenesis, mutagenesis and teratogenesis (Breimer 1988;Upton et al.1992; Nikula et al. 1995, IARC 2002; Eberhard et al. 2013; Comishet al.2014).

Radiation damages occur through collision of photon particles with atoms and molecules in cells which ionize to give rise to ions and free reactive radicals. Free radicals are believed to play a major role in more than sixty different health conditions including the ageing process, cancer and arthrosclerosis (Sanaa et al.2015). Gamma radiation

induced damage manifests itself in somatic and germ cells in a variety of ways for ex.non disjunction, non-duplication of chromosomes, DNA damage and repair (Boer et al., 1983; Guedeney et al.1989; Almodovar et al. 1994; Barnard et al. 2013). The consequential effect of this is characterized by mutation and cell cycle delay. Loss of reproductive abilities and even survival are the long term effects of these cellular and molecular pathologies (Hittleman et al.1980;Fowler1989;Sanaa et al.2015). Dividing and propagating cells are more vulnerable to radiation damage vis-a-vis non dividing cells. The bone marrow is highly susceptible to oxidative damage induced by irradiation(Umegaki and Ichikawa 1997; Sanaa et al.2015)

Cytogenetic damage caused by ionizing radiation is very well known (Krepinsky et al. 1983;Kadhim et al. 1995;Ottolenghi et al2001; Milacic2003). Gamma radiation and other types of ionizing radiation randomly disturb the morphology of chromosomes. However, the ultimate target in the mammalian cells is DNA base damage which leads to various forms of mutation. The main noticeable changes in chromosomes are their fragmentation, dicentric rings, gaps, break and translocation (Lambin 1994; Sarawarthy et al.2000; Milacic 2004).

Therefore, a careful perusal of the literature on the subject shows fragmented, controversial and incomplete information on the comparative aspect of the effect of different doses of gamma radiation on the mitotic index and chromosomal morphology .The present study is, therefore, carried out on the bone marrow cells of sexually mature adult male of Swiss albino mice to fill in some of these gaps.

MATERIALS AND METHODS

Procedure of radiation

The animals were restrained in position by tying rubber bands around the forelimb and hind limbs. They were exposed to single pulse of various doses of gamma radiation by Cobalt -60 camera. Radiation was applied to the abdominal region where the paired testes were located.

Sexually mature Swiss albino mice weighting 18 $\pm$2 gram were used in the present studies. Five groups were set up each having 5 mice.

Group 1:served as control, and were sham irradiated.
Group 2: were irradiated by 0.2Gy of γ radiations
Group 3: were irradiated by 0.4Gy of γ radiations
Group 4: were irradiated by 0.6Gy of γ radiations
Group 5: were irradiated by 0.8Gy of γ radiations

All experimental groups and control group sacrificed after 24 h after giving single dose of irradiation. These experiments were repeated twice. Control and irradiated mice were injected intraperitoneally 0.04% colchicines per 100 gm body weight. After one hour the animals were sacrificed by cervical dislocation. Femur were dissected out and their heads were cut. Bones were flushed with hypotonic solution and their contents collected in centrifuge tubes, which were incubated at 37^0 for 30 to 40 min. After incubation, tubes were centrifuged at 800 -1600 rpm for 10 min and their supernatant discarded. Freshly prepared fixative was added to the residue and this was centrifuged. This step was repeated 3-4 times for optimal washing of cell by fixative. The suspension of cells was further processed as follows:

Ultraclean chilled slides were held between fingers at 45^0. The suspension containing bone marrow cells were delivered drop by drop on tilted slide from a distance of 20-30 cm for effective breaking of cells and therefore the chromosomes spread clearly. The slide was then passed over sprit lamp to burn excess acetic acid. This warming was done quickly to avoid cooking of chromosomes.

The prepared slides were observed for morphology and mitotic index was calculated using the following formula:

$$\text{M.I.} = \left(\frac{A}{A+B}\right) \times 100$$

where, M.I = Mitotic index; A = Number of metaphase plate (dividing cell); B = Number of non-dividing cells.

RESULTS

Mice Irradiated by 0.20Gy

This was the lowest experimental dose of the study. The metaphase plates manifested the following abnormalities: The breaks and fragments were also observed which 0.933 were. Rings were also observed and the average mean value was estimated to be 1.067. Small number of dicentric chromosomes were also visualized and their average mean number was0.533. Other types of aberrations were not seen at this dose level. The mitotic index showed small decrement as compared to control and was 24%.

Mice Irradiated by 0.40Gy

This type of aberrations observed on this dose were fragments and breaks which numbered 1.600. The number of rings seen were 1.13. The incidence of dicentric chromosomes were calculated to be 0.8. The mitotic index was 20%.The other parameters of the observations were negative.

Mice irradiated by 0.60Gy

This type of aberrations increased in ratio of higher dose. The number of fragments and breaks were 1.66, rings were 1.6, and calculated dicentric chromosomes were 1.333. In addition to these aberrations aneuploidy was also observed which was computed to be 0.73. The mitotic index was estimated to be 19.6%.

Mice irradiated by 0.80Gy

This was the highest experimental dose of the present study. At this dose highest frequency of aberration were recorded. The fragments and breaks were 2.13. The average number of rings were computed to be 2.4.The frequency of dicentric chromosomes was 1.73. Aneuploidy was observed in large number of dividing cells and was estimated to be 1.13. Mitotic index was 17.68%.

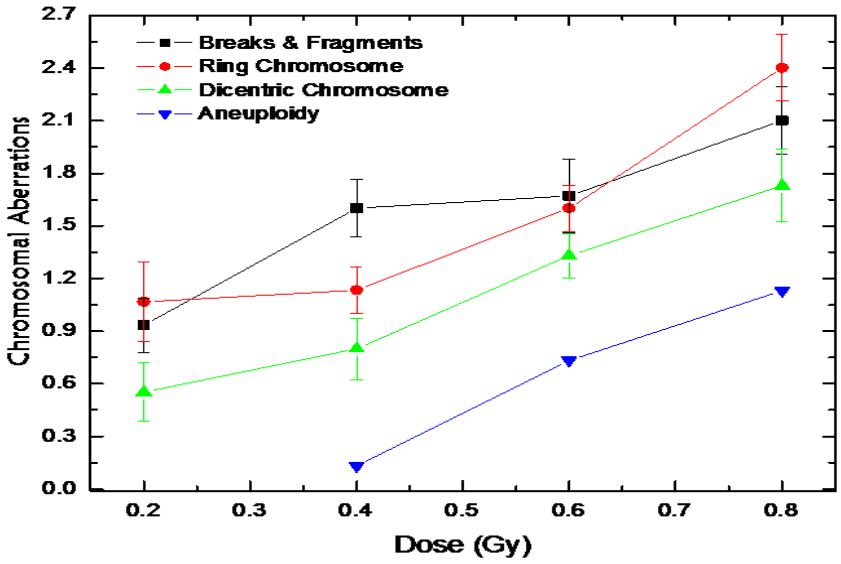

Fig.1 Various chromosomal aberrations on different doses of gamma radiation

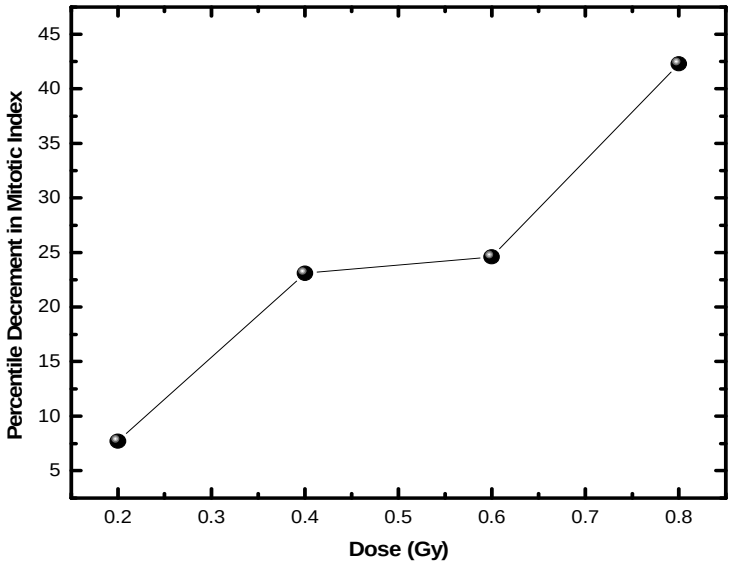

Fig.2 Total chromosomal aberrations on various doses of gamma radiation

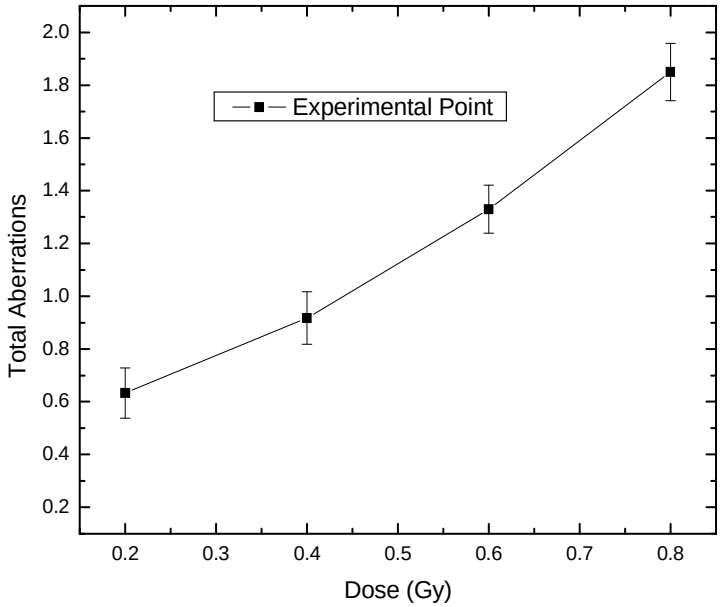

Fig. 3 Decremental impact on Mitotic Index of Swiss Albino Mice as increase the dose of radiation

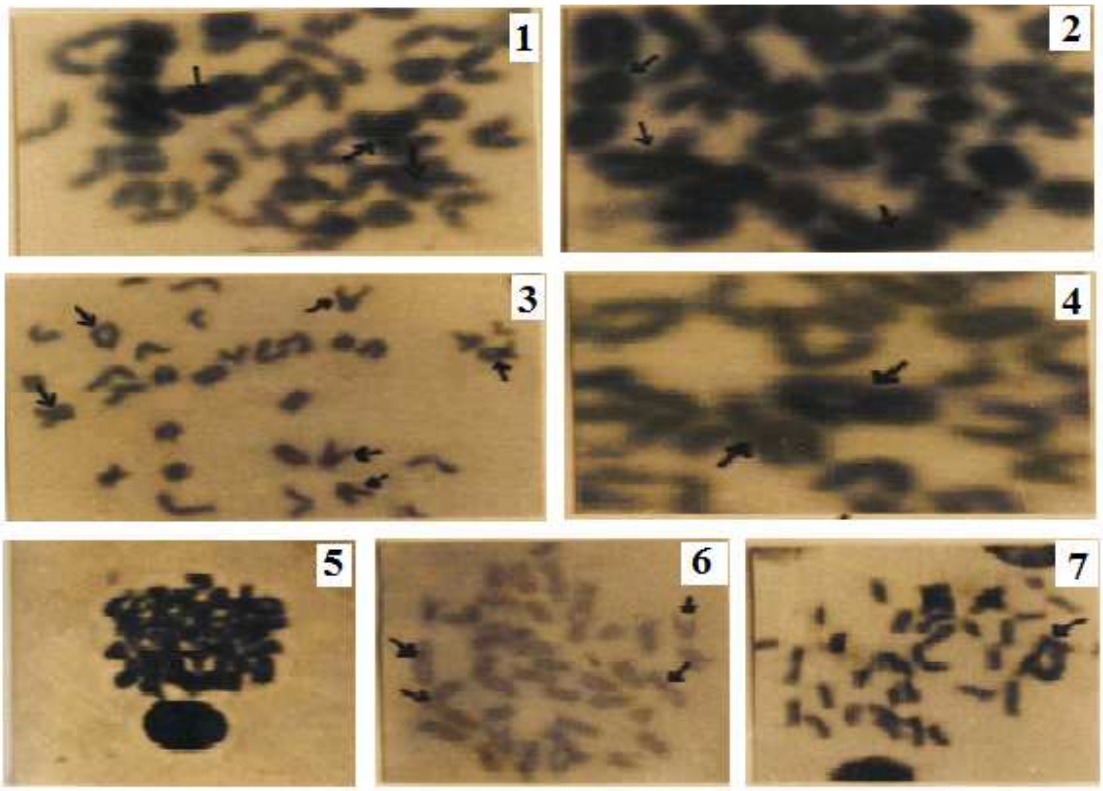

Fig. 4 Morphological changes observed in bone marrow chromosomes post administration of Gamma radiation

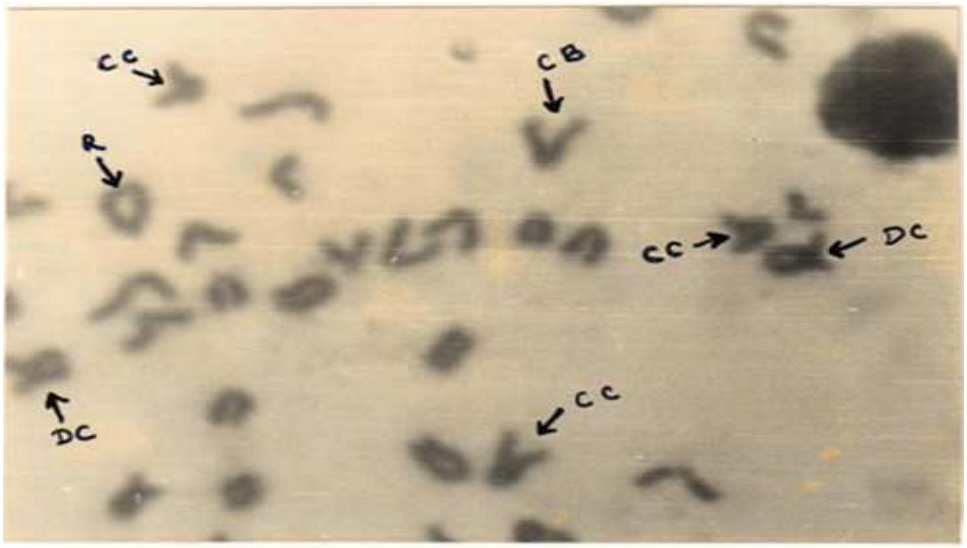

Fig. 5 Chromosomal aberrations induced by various doses of gamma radiation. Dicentric chromosomes (DC); Ring chromosomes (R); CB – chromatid break (CB); CC – chromatid constriction

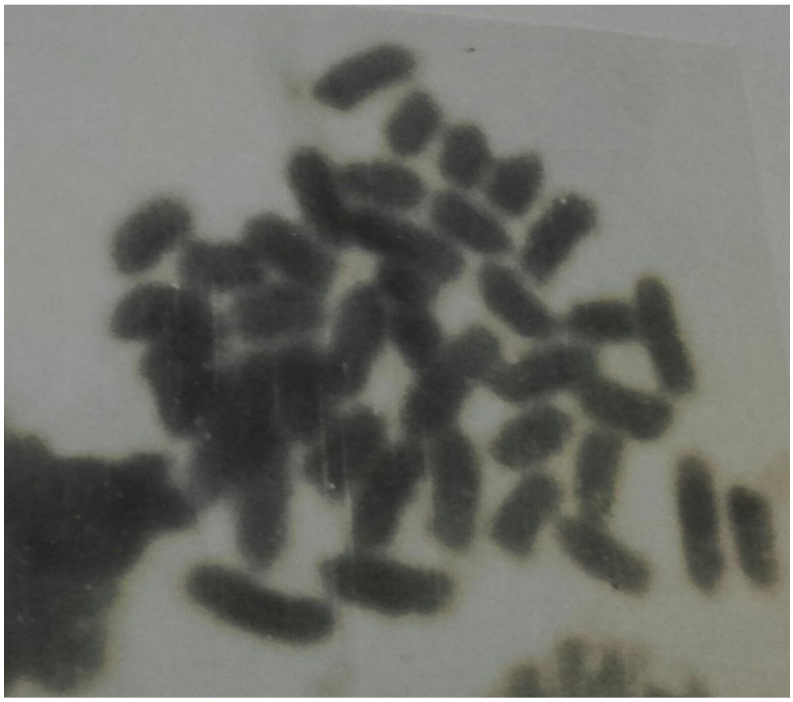

Fig. 6 Conventionally stained metaphase of Swiss albino mice showing aneuploidy

Control

This group of mice which was sham irradiated showed no abnormalities in their chromosome morphology and number. The mitotic index was computed to be 26%.The number (2n) of chromosomes was 40. Autosomes and sex chromosomes were clearly visible.

At the doses of 0.2 Gy, a variety of aberrations were observed fragments formation, appearance of rings, and formation of dicentric chromosomes by Robertsonian fusion. The average aforesaid abnormalities were greater in frequency as the dose increased i.e. 0.4, 0.6 and 0.8 Gy inspite of previous aberrations aneuploidy was also observed at the dose of 0.60 Gy and 0.80Gy. This shows that dividing cells of the bone marrow are severely affected at higher doses.

The Mitotic Index in irradiated mice gradually decreases. At the dose of 0.20Gy the percentile decrement was 7.69% vis-a-vis control. The dose of 0.40Gy, 0.60Gy, 0.80Gy, the computed values of percentile decrement were 23.07%, 24.61%, 42.30% as compared to control (considered as 100%).

DISCUSSION

In the present study it was observed that the aberration in morphology and mitotic index manifested a linear dose related decremental trend. This is compatible with the observation of Awa et al.,1971;Sofuni et al., 1978, Evans et al.,1980,Gupta and Umadevi 1986, Kligerman et al., 1988 Diener and Voglan,1988, Jagetia 1993 , Jain 1995 , Sarawarthy, R.et al 2000, IARC 2002, Ivancsits et al. 2003, Beels,et.al 2010,Roy L.et al.,2012), on a variety of placental as well as humans subjected to cancer therapies by using cobalt-60 gamma radiation. Higher number of abnormalities in peripheral blood lymphocytes at low dose (Umagaki, K. and Ichikania, T.1997) .Samarth, R.M,Kumar ,A.,(2003) also reprted chromosal aberrations in bone marrow of mice. These observations agree well with the abnormalities detected in bone marrow cells of mice in the present study. Sarawarthy, R.et al. 2000 observed dicentric chromosomes at low doses of 0.2 Gy to 0.5Gy.This data is supportive of the results of the present studies. Kovacs et al.1994 and Barnard et al.,2013 observed breaks and exchanges at the dose of 6Gy after 24 hours of post irradiated in humans. Lambin et al (1994) studied chromosomal aberrations in two human tumour cells (MeWo and HT29) using fluorescence (ionized radiation) and reported high radiosensitivity of MeWo than. HT29 cell. They observed break, fragments and translocation at dose from 0.25Gy to 5.0Gy. Somewhat similar

observations have been detected in the present study in the bone marrow cells of Swiss albino mice, although the cell type are different structurally , physiologically and metabolically.

Diener et al.,(1988) Saraswarthy, and Natrajan(2000) and Sanaa A.,et al.,(2015) observed dicentric chromosomes, acentric fragments. And ring chromosomes in radiation workers and patients suffering from Morbus Hodgkin disease, Xeroderma pigmentosum, Fanconi anemia patients, after radiation therapy. A linear relationship between aberrations and dose was observed by them. Similar results were reported in patients afflicted with ankylosing spondylitis by Buckton *et al.,(*1962*).*This is in the close agreement with the present findings in as far as aberrations are concerned after administration of gamma radiation.

Results of many studies on human lymphocytes have shown a significant decrease in aberrations when dose rate was lowered from 50 Rad/min to 0.3 R/min (Scott et al.,1970,Brewen and Luippold 1971, Lloyd et al.,1977, Ottolenghi et al, 2001; Beels, et al., 2010).The results relating to the low dose are compatible with the present findings on the bone marrow cells of Swiss albino mice.

Mahieu *et al.*,1994,Richardson and Jasin,2000 and Roy L et al.,2012 reported dicentric rings, translocations, chromatid and chromosomes fragments, chromatid gaps in patients of thyroid cancer after treating them with a dose of 1850 MBq of 131 Various types of chromosomal abnormalities have been detected in humans exposed to uranium, radiations emanating mines and from nuclear plants (Chernobyl) (Brandom et al.,1972 and Ostreicher, 1993)

Increased emphasis has been laid and efforts made to decode the enigmatic molecular mechanism(s) relating to stable type of chromosomal abnormalities. It has been stated that impairment of DNA organization; DNA sequence damage and DNA strand break induced by ionized radiation in mammals (Natrajan et al.,1986 ,Fornace 1992, Lagroye and Poncy 1997, Ding et al., 2000)

Breaks in the double strand of DNA designated as initial damage are known to be the cause of mutation. However, because of structural stability of DNA its repair and removal by cellular mechanism takes a long time (Zaideret al,1994; Barnard,et al.,2013).

It is of interest to record here the observation of Pohl-Ruling et al.,1983 and Klingerman *et al.,(*1988) who treated mouse and human peripheral blood lymphocytes by differential doses e.g.,1.0 , 2.0 , 3.0 ,4.0 Gy of ^{60}co- radiation. They found dicentric ring chromosome and deletion (in vitro as well as in vivo).This trend of aberration also manifested a linear relationship with the dose. Edward (1995) has reviewed the literature on this aspect and has concluded that the aforesaid response is made by all types of cells hitherto studied when challenged by different doses of gamma radiation.

In the present study the mitotic index was significantly altered vis-à-vis control. Such significant alterations have also been reported in other mammals including humans by Lajtha and Oliver 1961; Brooks1980; Hall 1988, Geard and Chenn, 1990 and 1994, Roy, L. et al.,2012).

Diener *et al.*,(1988) Thomson et al.,(1988) found decrement in cells number as the dose was increased in Morbus Hodgkin patients after radiation therapy. Thus the mitotic index was also influenced by ionizing radiations. This agrees with the present study although the treatment in this case is related to healthy bone marrow cells of mice.
Dose-related damage to the dividing cells has continued to attract the attention of many workers. Thus, Kligerman et al., (1988) showed this in mouse and human after ^{60}co gamma radiation. This report is supportive of the present observations on mice.

The questions of vulnerability and responsiveness of target cells to gamma radiation have been studied. In general, it has been stated that the proliferation kinetics of cells in certain organs with a fast turnover of cells such as the bone marrow, blood, skin and the gonads appeared to be the prime target (Liu, et al., 2006; Khan,et al., 2015). On the other hand, organs having slow turnover of cells like the kidneys, lungs, the heart and in muscles, the damage appears after some weeks or even months (Yi *et al.*,1994).

CONCLUSION

The result of the present study clearly indicates that the mitotic index of irradiated mice was severely altered and many aberrational changes occurred in the chromosome morphology. This may be due to disturbances/oscillations in the molecular mechanism/ interactions. Bone marrow cells showing such defective chromosomal morphology possibly may also suffer from attenuation of their genetic, physiological and biochemical mechanism(s). Thus, the current and rampant use of ionized radiation warrants further, in-depth investigation in view of long term genetic hazards.

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