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RESEARCH ARTICLE

Study of Some Biochemical and Molecular Changes Induced by Radiation Hormesis in Testicular Tissues of Male Rats

Rasha Yousef Mohamed Ibrahim* and Mona Ahmed Mahmoud Ghoneim
Department of Radioisotopes, Nuclear Research Centre, Atomic Energy Authority, Egypt.

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****Corresponding Author***

Rasha Yousef Mohamed Ibrahim

Abstract

With the development of modern nuclear science and technology as well as the peaceful use of atomic energy and radioisotopes, we examined the possible benefit effects of exposure of the whole body to low dose gamma irradiation for long term (radiation hormesis) on the status of some antioxidant defense system, molecular and histological changes in the rodent testicular tissues using two different low doses of γ irradiation. Thirty adult male albino rats were used in this study, which were divided into three groups of 10 rats each. The first group was maintained as control whereas groups two and three were exposed to fractionated 20 and 60 mGy whole body gamma radiation, respectively 3 days/week for four weeks (with accumulated doses 0.25 Gy and 0.72Gy, respectively). The biochemical: malondealdhyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and testosterone, molecular (Caspase-3 activity and double strand DNA breaks in testicular tissues by comet assay) and histological estimations were investigated. The results showed significant ($P<0.05$) increase in MDA level and caspase-3 activity, while a significant decrease ($P<0.05$) in superoxide dismutase (SOD) activity and reduced glutathione (GSH) level and testosterone level in both irradiated groups compared to control group. Moreover, irradiation treatment significantly increased the DNA damage of testes in a dose-dependent manner as observed by comet assay parameters compared to the control. It was concluded that the whole body exposure of animals to low doses gamma radiation did not detect any beneficial effects to radiation hormesis in testicular tissues which confirm sensitivity of testes to low doses of radiation.

Introduction

Due to the progressive development in all areas of science and technology in the world there are a growing number of various sources of radiation. Those include mobile communications, the development of new methods of medical diagnostics, space exploration, the creation of nuclear weapons, and the development of the nuclear industry and power that led to a serious threat to the environment and human health. As a result, today in some regions the total value from all techno genetic sources is comparable with the dose of background radiation from natural sources **(Nakamura et al., 2012).**

The health risk associated with low levels of ionizing radiation is still a matter of debate. A number of factors, such as non-target effects, adaptive responses and low-dose hypersensitivity, affect the long-term outcome of low-dose exposures **(Ropolo et al., 2012).**

After an exposure of biological systems to any stress situation, an adaptive response is usually observed. This is especially after low levels of exposure. Frequently a homeostatic response is found which leads to upwards and downwards regulations in order to reach the original physiological state again. These events are essential for

recovery and regeneration in life and can be observed on all organizational levels in nature (macromolecules, cells, tissues, organisms) **(Ostendorf and Seeber, 1997).**

During the past three decades, genomic, cellular, animal and human data have shown low-dose ionizing radiation, including acute doses up to 30 cGy, stimulate each component of the homeostatic antimutagenic control system of antioxidant prevention, enzymatic repair and immunologic and apoptotic removal of DNA alterations. On the other hand, high-dose ionizing radiation suppresses each of these antimutagenic protective components **(Pollycove, 2007).**

The biological effects of low-level radiation have attracted the attention of investigators for more than 20 years. The biological effects of low-level radiation on cellular metabolisms and defense systems sometimes called hormesis **(Feinendegen, 2005**) which led to increased immune responses and antioxidant capacity (**Lee et al., 2000**). However, **Liu et al. (2006)** reported that the exposure of the experimental animals to low-level radiation induces increased apoptosis in male germ cells.

During exposure to low-level doses (LD) of ionizing radiation (IR), the most of harmful effects are produced indirectly, through radiolysis of water and formation of reactive oxygen species (ROS). The antioxidant enzymes--superoxide dismutase (SOD): manganese SOD (MnSOD) and copper-zinc SOD (CuZnSOD), as well as glutathione (GSH), are the most important intracellular antioxidants in the metabolism of ROS. Overproduction of ROS challenges antioxidant enzymes (**Durović et al., 2008).**

ROS species are constantly produced in various cells and tissues. There concentrations are determined by the balance between their rates of production and rates of clearance by antioxidant enzymes and compounds. Living cells and tissues possess several mechanisms used for maintenance of the redox balance even after temporary exposure to increased concentrations of ROS, so–called redox homeostasis, which is maintained by antioxidant defense systems and their associated regulation pathways **(Dröge, 2002).**

However, when ROS is vigorously and persistently produced, the antioxidant response may be insufficient to reset the system to its original level of redox homeostasis, thereby resulting in a deregulated redox balance. Elevated ROS damages various proteins, membranes, and nucleic acids and consequently gives rise to apoptotic cell death. Uncontrolled ROS production is usually responsible for environmental, inflammatory, and mitochondrial oxidative stress. However, if the initial increase in ROS is relatively small, the antioxidant response may be sufficient to compensate for resetting the original balance. Subsequently, various redox-sensitive signaling pathways are activated, which in turn activate and repair the defense system. Thus physiological redox regulation can be attributed to a temporary increase in ROS and a temporary shift in the redox state toward more oxidative conditions. Ionizing radiation is the oxidative stress most widely studied for its low- dose effects. Low dose radiation has been reported to induce hormesis (**Upton, 2001)**, which is a beneficial stimulant effect of chronic low-dose radiation**.**

Hormetic and adaptive responses induced by low-level radiation in hematopoietic and immune systems have been observed, as shown by stimulatory effects on cell growth and resistance to subsequent radiation-induced cytogenetic damage. However, in terms of cell death by apoptosis, the effects of low-level radiation are controversial; some studies showed decreased apoptosis in response to low-level radiation while others showed increased apoptosis. This controversy may be related to the radiation doses or dose rates and also, more importantly, to the cell types. Testes are one of the most radiosensitive organs. The loss of male germ cells after exposure to ionizing radiation has been attributed to apoptosis **(Liu et al., 2006)**.

Apoptosis occurs under the direction of an active, intracellular death program that can be stimulated or inhibited by environmental agents (**Jacobson et al., 1997).** Under developmental regulation, apoptosis proceeds in response to: a) deprivation of survival factors, such as testosterone, b) activation by ligated death factors, or c) exposure to environmental stimuli, such as radiation, chemotherapeutic drugs, and ROS **(Sinha Hikim and Swerdloff, 1999).** By activating caspases, ROS may initiate the propagation of a series of reactions that ultimately trigger apoptosis. Additionally, apoptosis may be induced by cell injury or stress. The principal function of testicular apoptosis is to help maintain tissue homeostasis during spermatogenesis **(Martinčič et al., 2001).**

Testicular apoptosis serves to deplete excess germ cells and remove abnormal spermatozoa during normal spermatogenesis. Indeed, apoptosis eliminates 75% of germ cells before they become fully mature **(Martinčič et al., 2001)** and (**Shaha et al., 2010**). In this way, testicular apoptosis monitors germ cell population according to the support capacity of Sertoli cells (**Shaha et al., 2010).** While apoptosis is essential for maintaining testicular homeostasis during spermatogenesis, inappropriately occurring apoptosis has been linked to suboptimal male reproductive function **(Martinčič et al., 2001).** Excessive apoptosis results from impaired regulation or improper activation, and can affect spermatogenesis and even lead to infertility **(Lizama et al., 2007).** Therefore, the aim of the present study is to investigate radiation hormesis and radiosenstivity of testicular tissues of male rodents by evaluating the status of some antioxidant defense system, molecular and histological changes in testes tissues.

Materials and Methods

Irradiation

Whole-body gamma-irradiation was performed at the Middle Eastern Regional Radioisotopes Center for Arab Countries, Dokki, Giza, Egypt, using Co^{60} source. Animals were irradiated with two fractionated doses of 20 mGy and 60 mGy delivered 3 days a week for 4 weeks at dose rate (36 cGy/min).

Experimental design

Thirty male albino rats (150-175 g weight) obtained from the Animal House Colony of the National Cancer Institute, Cairo, Egypt, were used in the present study. The animals were randomized and housed under ambient room- temperature and relative humidity conditions, a commercial diet and water were provided add libitum. All experimental animals were performed following the Principles of laboratory animal care (NIH publication no. 85-23, revised 1985), as well as specific local institutional laws for protection of animals under the supervision of authorized investigators. Animals were randomly divided into three groups each of 10 rats. The first group (GI) served as normal control. The second group (GII) and the third group (GIII) were exposed to fractionated doses (20 mGy) and (60 mGy), respectively 3 days a week for 4 weeks whole body exposure.

Biochemical Analysis

Preparation of homogenate tissue.

The excised testicular tissue was washed with distal water for the removal of blood, and later the fatty parts were removed. Tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0. 1 mM ethylenediamine tetra-acetic acid (EDTA). The supernatant was separated by means of centrifugation at 1000 g for 20 min at 4 C. The supernatant were used for analyses of the antioxidant enzymes, estimation of lipid peroxidation and assay of antioxidant enzymes in testes. Tissue samples of all animals in the experimental groups were subjected to the following determinations. Testicular lipid peroxide measured as malondealdehyde concentration was evaluated according to **Wills** (**1987)**. Determination of antioxidant status occurred by measuring reduced glutathione (GSH) concentration and superoxide dismutase (SOD) activity in testicular tissues according to **Ellman (1959)** and **Misra and Fridovich (1972),** respectively. Measurement of testosterone hormone in testicular tissues was done according to **Ito et al. (2011)**.

Molecular Detections

1-Detection of Caspase-3 activity using real time PCR (RT–PCR)

RNA extraction

Total RNA was isolated from testis tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer's instruction. The purity (A260/A280 ratio) and the concentration of RNA were obtained using spectrophotometry (GeneQuant 1300, Uppsala, Sweden). RNA quality was confirmed by gel electrophoresis. cDNAsynthesisFirst-strand cDNA was synthesized from 4 µg of total RNA using an Oligo(dT)12-18 primer and Superscript™ II RNase Reverse Transcriptase. This mixture was incubated at 42C for 1 h, the kit was supplied by SuperScript Choice System (Life Technologies, Breda, the Netherlands).

Real-time quantitative polymerase chain reaction (PCR)

Real-time PCR (RT-PCR) amplification was carried out using 10 µL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA USA), equivalent to 8 ng of reverse-transcribed RNA and 300 nM primers, the sequences of PCR primer pairs used for each gene are shown in Table 1. Reactions were run on an ABI PRISM 7900 HT detection system (Applied Biosystems). PCR reactions consist of 95 C for 10 min (1 cycle), 94 C for 15 s, and 60 C for 1 min (40 cycles). Data were analyzed with the ABI Prism sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the beta actin genes according to **Livak and Schmittgen (2001).**

Table (1):The primer used for the amplification for real time quantitative RT-PCR detection.

Primer	Sequence
Caspase-3	FORWARD: 5'- ATGGACAACAACGAAACCTC-3 ' REVERSE: : 5'- TTAGTGATAAAAGTACAGTTCTT -3 ' according to gene bank accession number A062449

2- Detection of DNA damage in testicular tissues by the comet assay:

The comet assay was performed as described by **Singh et al. (1988)**. 0.5 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. Cell suspension (100 µl) was mixed with 600 µl of low-melting agarose (0.8% in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Staining with ethidium bromide (EtBr) 20µg/ml at 4 C. The observation was with the samples still humid, the DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope (with excitation filter 420-490nm [issue 510 nm]). The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr-stained DNA using a 40x objective on a fluorescent microscope.

Although any image analysis system may be suitable for the quantitation of SCGE data, we use a Comet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK) linked to a CCD camera to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculates tail moment. Generally, 50 to 100 randomly selected cells are analyzed per sample.

Histopathological Investigation:

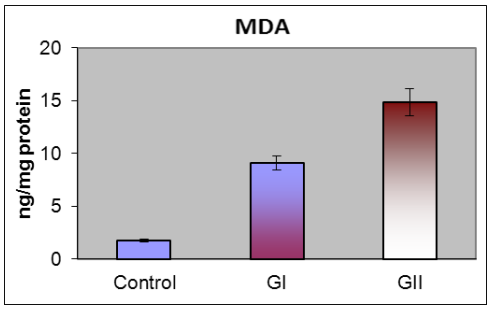
Autopsy samples were taken from the testes of rats in different groups and fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination, then examination was done through the light electric microscope **(Banchroft et al., 1996)**.

Statistical Analysis:

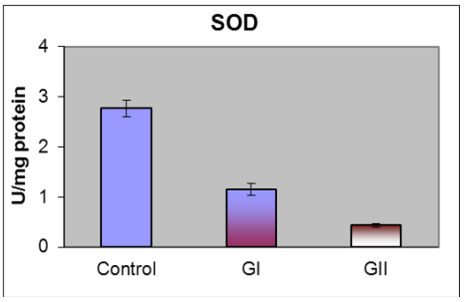
The data were statistically analyzed according to Steel et al. (1997) using SPSS computer Program (v.17). The results were presented as (mean ± SE). The differences between mean values were determined by analysis of variance (ANOVA test), followed by Waller-Duncan k- ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P \leq 0.05$.

Results:

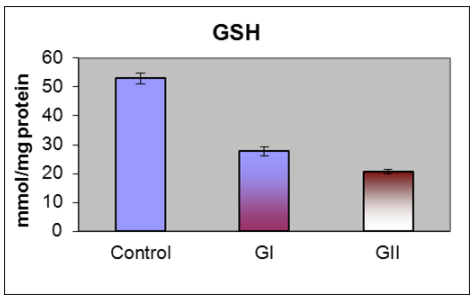
Figure (1:A) depict the level of MDA in the testis tissues of normal and experimental groups, low dose of γ-irradiation induced a significant increase ($p<0.05$) in the level of MDA of group II (GII) compared to control (GI). The elevation in the level of MDA continued in GIII throughout the designated duration of the experiment.



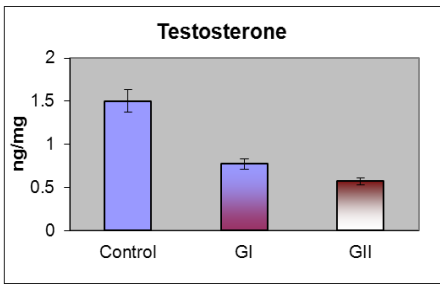
(B)



(A)



(C)



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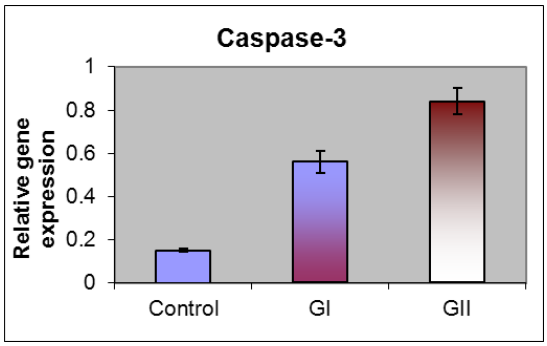


(E)

Fig.(1): Effect of whole body γ- irradiation with two different low doses on Lipid peroxidation (A), SOD activity (B), GSH level (C), Testosterone (D) and caspase-3 activity (E) in testicular tissues. Values are (means ±SE) P<0.05 vs. to control.

Results indicated that SOD activity and GSH level in different experimental groups were significantly decreased ($p<0.05$) in testicular tissues of rats treated with two different low doses of γ-irradiation when compared to control (GI) (Figures 1: B&C).Also, Irradiation stress resulted in testis injury in rats as manifested by significant decreases ($P<0.05$) in the activity of the antioxidant enzyme SOD and GSH levels in GIII compared to both GI and GII groups.

The whole body γ-irradiation of animals to low doses are shown in figure (1:D). A significant decrease ($P<0.05$) in the level of testosterone in testicular tissues was observed in group II as compared to normal control group and continued to decrease in group III as compared to group II and normal control group.

Testicular apoptosis in this study was detected by measuring caspase-3 activity. Data presented in (Fig. 1E) showed higher values in the activity in group II and group III, (0.56 ± 0.045) and (0.84 ± 0.060), respectively as compared to the value of normal control group (0.15 ± 0.005).

DNA damage detected in testicular tissues by the comet assay:

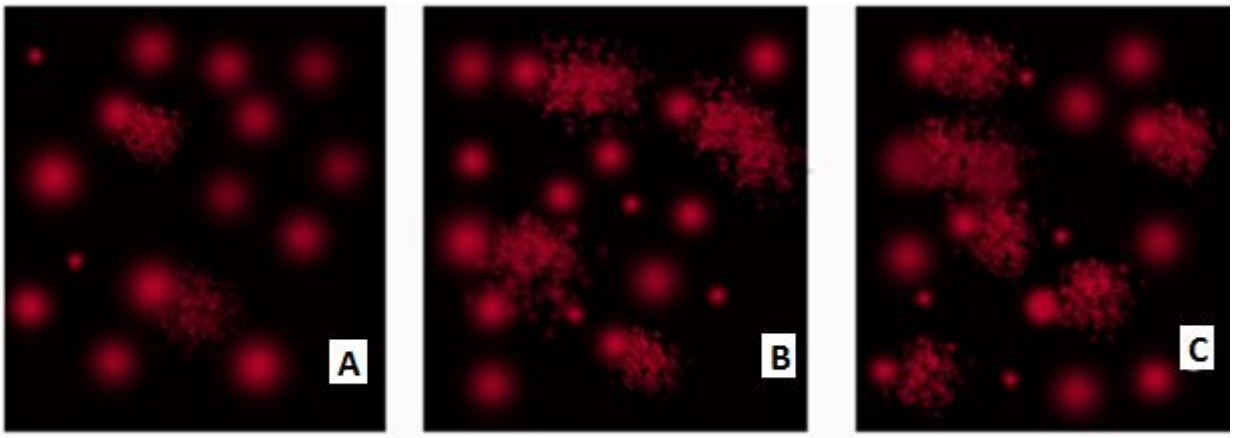
The effects of γ- irradiation on oxygen-radical-generated DNA damage in rat testes have been investigated using the comet assay, which allows the detection of DNA alterations of diverse types, such as double-strand breaks, single-strand breaks, alkali-labile sites, incomplete repair sites, and cross-links. Following single-cell electrophoresis, the lengths of the comets (DNA tails) depended on the effect of irradiation stress in experimental rats, longer tails indicating more DNA damage. Figures2 (A, B &C) show representative results for the migration of DNA from the organ examined for each treatment group. The results are summarized in Table (2). Control group testes of rat showed no tails (Fig. 2A). Tails were evident after 4 weeks exposure to 20 mGy and 60 mGy γ-irradiation, respectively. The mean value of tail length in the irradiation stress groups increased rapidly and significantly compared with their controls, and more DNA damage was observed in the tail when compared with control group (Fig. 2 B & C). Our results indicated that the levels of DNA degradation (comet tail length) were dose-dependent.

Histopathological Examinations:

As shown in Figure (3A), the testes of normal control rats revealed no histopathological alteration and the normal histological structure of the mature active seminiferous tubules(S) with complete spermatogenic series were recorded in (Fig. 3A). By contrast, in group (II) which was exposed to 20 mGy, some of the seminiferous tubules showed degenerative change(ds) with absence of spermatogenic series in the lumen associated with hyalinization(h) in the interstitial stroma (Figure 3B). While group (III), which was exposed to 60mGy, shows degeneration in most of the seminiferous tubules with absence of spermatogenic series and appearance of giant spermatogonial cells (arrow) in the lumen as well as hyalinization in the interstitial stroma (Figure 3C).

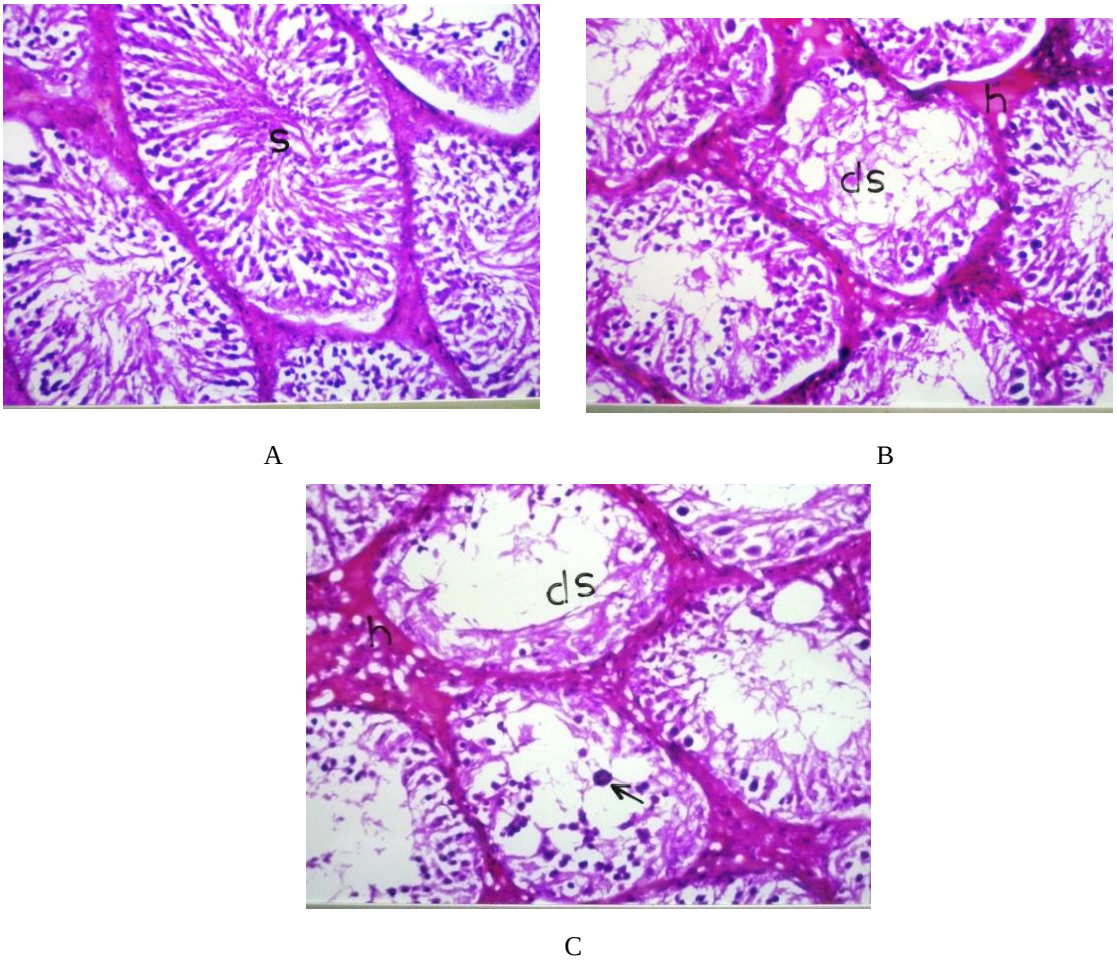
Table (2): The parameters of comet assay in thetesticular tissues of radiated and control groups.

parameters / Groups	% Tailed	% Untailed	µm Tail length	Tail DNA%	Unit Tail moment
Group I	**12**	**88**	**2.51**	**2.631**	**6.604**
Group II	**9**	**91**	**3.44**	**2.69**	**9.254**
Group III	**15**	**85**	**3.95**	**3.56**	**14.062**



Group I **Group II** **Group III**

Fig.(2): Effect of whole body exposure to γ- irradiation with two different low doses for four weeks on DNA damage in testicular tissues using comet assay demonstrating the comet tail of the affected nuclei compared to normal control.



A B

C

Fig.(3):Photomicrographs of test sections: (A) Group I, Control, (B) Group II, exposed to 20mGy and (C) Group III, exposed to 60 mGy.

Discussion

In the biological system, apart from normal metabolism, one of the active sources of oxygen radicals is the ionizing radiation. Cells can be injured and even killed under the most serious conditions of reactive oxygen species (ROS) gets under controlled by the cellular antioxidants. Our results demonstrated that whole body exposure to two fractionated doses (20 mGy, 60 mGy) of γ-radiation caused a significant increase in MDA level, whereas the activity of antioxidant enzyme SOD and antioxidant molecular level GSH were markedly decreased in testis tissues. Radiation induced ROS and free radicals react with the molecules of cell membranes and induce lipid peroxidation products (MDA) , which play an important role indirect biological damage such as mutagenic and carcinogenic damage (**West** and **Marnetti**, **2006; Poli et al**., **2008).**

Liu (1998) reported that low dose radiation could up regulate the defense and adaptive responses both in human population and animal models**.** The current study declared that although very small doses of γ- radiation oxidative imbalance in testicular tissues can induced. However, the results obtained contradict with earlier studies, which report decrease in lipid peroxidation levels in various rat organs and rabbit brain on exposure of the animals to low dose γ- radiation **(Yamaoka et al., 1991).** This variation in the formation of lipid peroxidation could be due to species difference of the animal model, the nature of the radiation source, the radiation dose, the dose rate and the time selected for measurement of various biochemical parameters **(Liu, 2011).**

The testes perform two vital, high energy-demanding functions, namely, spermatogenesis and steroidogenesis. In the testes, spermatogenesis and steroidogenesis occur within seminiferous tubules and the interstitium, respectively. These two compartments are morphologically distinct but are functionally connected **(Huleihel** and **Lunenfeld, 2004**). During spermatogenesis, a complex, interdependent population of undifferentiated

germ cells multiplies and differentiates to form spermatozoa. In the seminiferous epithelium, the germ cells are sequentially organized from the base of the tubule to the lumen, signifying the various stages of development. Several intratesticular and extra-testicular regulatory processes are involved in the regulation of normal spermatogenesis. The ROS that are generated during normal testicular function also play an important role in regulating the function of the testis. Although ROS are known to have damaging effects, controlled low levels of ROS play a beneficial role in normal testicular function. However, the production of ROS in the testis is primarily associated with phagocytic leukocytes in the semen **(Aitken and West, 1990**). Other cell types such as developing germ cells and spermatids are also a ready source of ROS **(Fisher and Aitken, 1997).** Wealth of evidence indicates ROS that are produced by sperm participate in the signal transduction mechanism that promotes sperm capacitation, the acrosome reaction and sperm maturation **(Ford, 2004**). However, increased levels of ROS can be detrimental to testicular function. To overcome this, the testis is equipped with very a potent antioxidant system that protects it from the damaging effects of ROS. The glutathione family of proteins, superoxide dismutase, catalase and several non-enzymatic antioxidants, all help the testis by counteracting any oxidative impact **(Aitken** and **Roman, 2008).** However, over stress represented in exposure to environmental toxicants or ionizing radiation has been shown to impair the pro-oxidant/antioxidant balance in the testis and thereby hamper testicular function, this results is consistent with **Saradha** and **Mathur (2006).** Additionally, the spermatogenic process can serve as both a source and a target of ROS.

In the present study, the effects of whole body exposure to low doses γ- radiation on male testicular tissues in terms of apoptosis were examined; the results showed that low-level radiation in the dose range of 20 mGy induced significant increases in apoptosis, with the maximal effect at 60 mGy. The results were in consistent with **Liu et al. (2006)** who reported that hormetic and adaptive responses induced by low-level radiation in hematopoietic and immune systems have been observed, as shown by stimulatory effects on cell growth and resistance to subsequent radiation-induced cytogenetic damage. However, in terms of cell death by apoptosis, the effects of low-level radiation are controversial; some studies showed decreased apoptosis in response to low-level radiation while others showed increased apoptosis. This controversy may be related to the radiation doses or dose rates and also, more importantly, to the cell types. Testes are one of the most radiosensitive organs. The loss of male germ cells after exposure to ionizing radiation has been attributed to apoptosis.

Erkkilä et al. (**1999)** reported that, the ROS that are produced during spermatogenesis are involved in the regulation of apoptosis within the testis. Testicular apoptosis occurs during differentiation of germ cells, which serves to adjust the number of germ cells in the testis according to **Rodriguez et al.** (**1997).** Testicular apoptosis occurs continuously throughout spermatogenesis, and both the intrinsic and extrinsic apoptotic pathways have been shown to play regulatory roles (**Tripathi et al., 2009).** The intrinsic (or mitochondrial) pathway involves various pro-apoptotic and anti-apoptotic proteins that recruit and activate the caspase cascade to induce apoptosis. The extrinsic pathway is mediated through Fas receptor (Fas) and Fas ligand (FasL) together with caspase proteins. Sertoli cells express Fas ligand, which signals the killing of Fas-expressing germ cells, thereby limiting the number of germ cells according to **Moreno et al. (2006),** and various factors such as the withdrawal of growth factors, radiation and oxidative stress trigger apoptosis in the testis. So, as the ROS increased inside the testes the apoptosis increased depending on stress and absorbed dose.

The immediate endocrine environment of the testes has a major impact on the antioxidant status of this organ. Exposure to two different low-doses γ- radiation diminish the intratesticular concentration of testosterone, The free radicals then attack the germ cells within the seminiferous tubules leading to extensive apoptosis and the disruption of spermatogenesis. The fact that inhibition of the P450 cholesterol side-chain cleavage induces extensive lipid peroxidation in the testis supports this contention (**Peltola et al., 1996).** Interestingly, over-stimulation of the Leydig cells by chronic exposure also stimulates high levels of ROS production from these cells, that in turn stimulate lipid peroxidation, reduction in antioxidant enzyme activities, germ cell apoptosis and the consequential disruption of spermatogenesis. This is consistent with **Gautam et al. (2007).** The involvement of the thyroid gland in the control of testicular function, a stable redox environment depends on an appropriately balanced gonadotrophic support, either hyper- or hypo-gonadotrophism will induce a state of oxidative stress in the testis (**Aitken** and **Roman, 2008**).

The present study has demonstrated an elevation in the percentages of tailed DNA and tail amount of testicular tissue after four weeks of exposure; it is well known that the testis is characterized by very high rates of cell proliferation. Since developing testicular sperm need supportive Sertoli cells, an overabundance of cell proliferation requires elimination of excess germ cells by apoptosis. One characteristic of apoptosis in eukaryotic cells is endonuclease cutting of DNA into discrete sizes **(Sakkas et al., 2004**).This may explain the presence of low level of DNA fragmentation among control of the present study. The presence of high rate of DNA fragmentation may be related to oxidative stress that occurs during exposure to radiation in the groups. This is in accordance with

Yamanaka et al. (2000), who related the testicular tissue damage during vasectomy to reactive oxygen species (ROS) exposure. Oxidative stress may significantly impair spermatogenesis and sperm function, which may lead to male infertility **(Agarwal et al., 2008**).

Our study demonstrates that rats treated by two different low doses of γ- irradiation, induced many histopathological and biochemical altered changes in testes tissue show degenerative changes and gonadal insufficiency. Both testicular histopathological abnormalities and low levels of testosterone hormone have been observed in the exposed rats. These alterations resemble those reported in experimental models of testicular damage, such as chronic testicular ischemia **(Al-Jahdali** and **Bisher, 2007; Hanafi , 2012).**

According to **Withers et al. (1974)** changes in weight and size of the irradiated testis is related more to depletion and regeneration of much more numerous cells in various stages of differentiation. In the present study, γ-irradiation treatments represented an apoptosis in the germ cells and absence of spermatogenic series, consistent with the finding of **Kangasniemi et al. (1996)** who proposed after exposure to low doses of gamma rays differentiating spermatogonia are killed. The depletion in spermatogonia resulted in a reduction in subsequent spermatozoa. Also, **Konoplia et al. (1996)** examined the microscopy morphological characteristics of Sertoli cells, Leydig's cells and other populations of testicular cells after prolonged exposure to low dose whole-body gamma-irradiation. They suggest that, the existence of gamma-sensor in brain of mammals that involved on hypothalamic-pituitary-testicular levels in realisation of radiation stress suppression of Sertoli cell functions at a relatively "low" (0.1-0.5 Gy) doses by means of hypothalamic releasing factors.

Conclusion:

The findings of this study demonstrate that no beneficial effects was noticed in testicular tissues after exposure to low doses of γ- irradiation for long term (radiation hormesis), so hormetic effects are weak and inconsistent, and a consensus is lacking on how hormesis should be defined and quantified. Also the study proved that testicular tissue is very sensitive to low doses of ionizing radiation.

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