PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

AVALIAÇÃO DO PAPEL DA VITAMINA C COMO AGENTE PROTETOR DE RADIAÇÃO UTILIZANDO γ-H2AX COMO SINALIZAÇÃO DE DANOS AO DNA EM TESTÍCULOS IRRADIADOS DE RATOS

EVALUATION THE ROLE OF VITAMIN C AS A RADIATION PROTECTIVE AGENT USING γ -H2AX FOR SIGNALING OF DNA DAMAGE ON IRRADIATED MICE TESTIS

تقييم دور فيتامين C كعامل وقائي من الإشعاع بإستخدام γ -H2AX للإشارة إلى تلف الحمض النووي على خصى الفئران المشعة

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RESUMO

Uma característica interessante da radiação ionizante, especialmente os raios gama e dos raios-X, como indutores de dano ao DNA, é a variedade de lesões que induzem. Os focos γ-H2AX são documentados para representar quebras de fita dupla de DNA (DSBs) como um biomarcador para danos induzidos por radiação. Desenho do estudo, 42 ratos machos adultos Albino BALB/c, foram divididos aleatoriamente em 6 grupos de sete ratos cada. O Grupo 1 recebeu uma solução salina padrão também não tratada, não exposto à radiação. Os ratos do grupo 2 receberam vitamina C (200 mg/kg.dia) intraperitoneal (i.p.) injetada durante 8 dias, sem radiação. Grupo 3, controle exposto à radiação gama. O grupo 4, controle, foi exposto à radiação de raios-X. Os camundongos do grupo 5 foram administrados com vitamina C na mesma dose do grupo 2 por 8 dias, depois expostos a 4 Gy de raios gama. O grupo 6 foi administrado com uma dose de vitamina C no mesmo período anterior e, em seguida, exposto a 4 Gy de raios-X. Todos os grupos foram sacrificados por luxação cervical em 1, 3 e 24 h. Os tecidos dos testículos dos ratos foram coletados pós-iradiação. Ocorreu uma diferença significativa ($P < 0,05$) entre o grupo de vitamina C com um grupo de controle exposto a raios-X e raios gama na formação de focos, mas não houve diferença significativa ($P > 0,05$) entre raios gama e raios-X para os grupos controle e vitamina C. Os resultados demonstram que a vitamina C é um bom agente radioprotetor para os tecidos dos testículos de ratos; o efeito dos raios-X e gama teveram quase os mesmos resultados nos tecidos dos testículo de camundongos com a mesma dose.

Palavras-chave: *Radiação ionizante, DSBs, Vitamina C, focos γ-H2AX*.

ABSTRACT

An interesting feature of ionizing radiation, especially Gamma and X-rays as a DNA damaging factor is the range of lesions it induces. γ-H2AX foci are documented to represent DNA double-strand breaks (DSBs) as a biomarker for radiation-induced damage. Study design 42 adult male mice Albino BALB/c, had been divided randomly into 6 groups of seven mice each. Group 1 received a standard saline solution untreated also, do not expose to radiation. Group 2 mice received vitamin C (VC) (200 mg/kg.day) intra-peritoneal (i.p.) injected for 8 days without radiation. Group 3 control was exposed to γ-radiation. Group 4 control was exposed to X-ray radiation. Group 5 mice had been administrated with vitamin C in the identical dose of group 2 for 8 days, then exposed to (4 Gy) of γ-ray. Group 6 was administrated with vitamin dose in the same above and the same period, then exposed to (4 Gy) of X-ray. All groups had been sacrificed by cervical dislocation at (1, 3, and 24 h). Post radiation testis mice tissues were collected. A significant difference ($P < 0.05$) between the group of vitamin C and with a control group exposed to both (γ, X-rays) in foci forming, but there is no significant difference ($P > 0.05$) between γ and X- rays for the control and vitamin C groups. The results demonstrate that vitamin C is a good radioprotective agent for testis mice tissues; the effect of (γ and X-rays) had almost the same results on the mice

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testicle tissues with the same dose.

Keywords: *Ionizing radiation, DSBs, Vitamin C, γH2AX foci.*

الملخص

السمة المدهشة للإشعاع المؤين، وخاصة أشعة جاما والأشعة السينية كعامل ضار للحمض النووي، هو مدى الآفات التي يسببها. تم توثيق بؤر γ-H2AX لتمثيل كسور الحمض النووي المزدوجة (DSBs) كمؤشر حيوي للضرر الناجم عن الإشعاع. في هذه الدراسة تم اختبار 42 من الذكور البالغين من نوع Albino BALB/c، وقد تم تقسيمها بشكل عشوائي الى 6 مجموعات تحتوي كل مجموعة على سبع فئران. المجموعة 1 تلقت محلول ملحي فيزيائي بدون معالجة غير معرضة للأشعاع. اما المجموعة الثانية فأن الفئران حُقنت داخل الصفاق بفيتامين C بجرعة (200 ملغم/كغم. يوم) لثمان أيام وبدون أشعاع. المجموعة الثالثة عُرضت الى أشعة جاما كمجموعة ضابطة. المجموعة الرابعة عُرضت الى الاشعة السينية كمجموعة ضابطة. تم إعطاء فئران المجموعة 5 فيتامين C في نفس جرعة المجموعة 2 لمدة 8 أيام، ثم تم تعريضها لـ (4 Gy) من أشعة جاما. أُعطيت المجموعة 6 جرعة فيتامين C في نفس الجرعة أعلاه ونفس الفترة، ثم عُرضت (4 Gy) من الأشعة السينية. بعد انتهاء التجربة تم قتل جميع المجموعات من خلال خلع الفقرات العنقية بعد (1، 3، و24 ساعة) من التشعيع. ثم جُمعت أنسجة الفئران بعد التشعيع. تُشير النتائج الى فروق ذات دلالة إحصائية ($P < 0.05$) بين مجموعة فيتامين C والمجموعة الضابطة التي عُرضت لكل من (أشعة جاما والاشعة السينية) في تكوين البؤر، ولكن لا توجد فروق معنوية ($P < 0.05$) بين مجاميع فيتامين C. أظهرت النتائج أن فيتامين C عامل جيد للوقاية من الاشعاع لأنسجة خصى الفئران؛ تأثير (أشعة جاما والاشعة السينية) له نفس النتائج تقريبا على انسجة خصى الفئران وبنفس الجرعة.

الكلمات المفتاحية. *الإشعاع المؤين، DSBs، فيتامين C ، بؤر γH2A .*

1. INTRODUCTION:

Ionizing radiation is widely used in medical diagnostics (cancer) and industrial applications (Smith *et al.,* 2017). Eventually, the human being is exposed to ionizing radiation from natural sources from cosmic rays and radioactive substances. Also, high-altitude flights and terrorism (nuclear weapons) are sources of radiation that certain populations are exposed to risk (Löbrich *et al.,* 2005).

Radiation can have direct and indirect biological effects (Hall *et al.,* 2016). If the biological material absorbs the X-ray or γ energy, the radiation can interact directly in the cell tissue. The ionization or excitation of atoms results in chain reactions culminating in biological damage (Hall *et al.,* 2016). Ionizing radiation damages the tumor microenvironment by preventing cell growth (Wu *et al.,* 2017). The radiation energy can be absorbed in tissue and induce chemical and biological changes in the cell. Double-Strand DNA Breaks are widely recognized as the most biologically important lesion that causes cancer and genetic diseases (Rothkamm and Löbrich, 2003). The Double-Strand Breaks (DSBs) are considered to be the most important DNA lesions caused by ionizing radiation. These DNA lesions are typically repaired efficiently; however, DSB miss-repair can lead to chromosomal trans-sites (Kuefner *et al.,* 2012).

DSBs can be generated indirectly through water radiolysis (decomposition of water molecules due to ionizing radiation), creating reactive chemical species damaging nucleic acids, proteins, and lipids. (Azzam *et al.,* 2012). When ionizing radiation (γ or X-ray) is directed at an object, some photons are absorbed or scattered, while others completely penetrate the object. Penetration can be expressed as the fraction of radiation passing through the object (Anderson and Warner, 1976). Cellular tissues are made up of 80% water, so the main damage is caused by the formation of free radicals produced by radiation (Shrishrimal *et al.,* 2019).

The impacts of ionizing radiation on the biological system are the generation of Reactive Oxygen Species (ROS) containing superoxide anion, peroxides, and hydroxyl. (Azzam *et al.,* 2012; Cai *et al.,* 2001). Free radicals can be defined as molecular species that contain an unpaired electron in an atomic orbital. The presence of this unpaired electron produces unstable and highly reactive radicals (Gutteridgea, 1994). A healthy organism can neutralize the formation of large amounts of harmful molecules. However, some conditions lead to a cellular imbalance resulting in oxidative stress (is an imbalance between free radicals and antioxidants in body) (Wojtunik-Kulesza *et al.,* 2016; Tan *et al.,* 2018).

The oxidative damage observed in the DNA causes mutagenesis and carcinogenesis. Cellular damage can be observed by radiation of high linear energy transfer (LET) (particle α) and radiation of low LET (γ and X-rays). The main difference between high and low LET is the appearance of free radicals during water radiolysis (Cai *et al.,* 2001).

Cytotoxic agents (ionizing radiation) can induce double-strand breaks with damage to DNA. This behavior is followed by a cellular response called histone phosphorylation (H2AX). This histone protein belongs to the H2A family. H2AX

histone, when phosphorylated, forms foci of H2AX in the lesion sites. Histone, when phosphorylated, is called γ-H2AX and is used as a biomarker for radiation-induced damage (Bassing *et al.,* 2002; Kuo and Yang, 2008; Cleaver *et al.,* 2011).

Several studies have analyzed γ-H2AX in tissue samples in mice. The objective of the research was to determine the radiation during several treatments (Rothkamm and Horn, 2009). DNA damage plays a significant role in developing atherosclerosis and other degenerative diseases, including some form of autism, Alzheimer's, Parkinson's, and cancer. The risk of radiation injuries is due to the doses received radiation (low or high LET) and the type of cell (González *et al.,* 2018; Mirończuk-Chodakowska *et al.,* 2018).

New antioxidants (water-soluble molecules) have been used to decrease radiation damage (Sukhotnik *et al.,* 2018). Small, enzymatic molecules have been tested to reduce cytotoxicity and long-term oxidative damage (Okunieff *et al.,* 2008). Antioxidants act in an oxidative series with many stages through lipid peroxidation in cell membranes (Gutteridgea, 1994). Vitamin C (VC) is the most important water-soluble antioxidant (Lakshmi and Kesavan, 1993). In addition to being considered an essential micronutrient, it can donate electrons (Carr and Maggini, 2017).

Vitamin C acts as precursors to enzymes, protects food substances from the oxidative cycle, aids in the absorption of food in the intestine, and protects oxidizing agents in the blood (Rahayu *et al.,* 2019). Vitamin C protects DNA against radiation damage (Cai *et al.,* 2001). Thus, changes in the structure of DNA, caused by ascorbic acid, are closely related to its ability to defend against DSBs (Yoshikawa *et al.,* 2006). Vitamin C can eliminate ROS even before the proliferation of macromolecules (*Rahayu et al., 2019*). Another function of vitamin C is protecting lipid membranes from oxidative damage (González *et al.,* 2018).

Unlike most mammals, humans do not have the capacity to produce vitamin C. Therefore, and it must be obtained through food (Taş *et al.,* 2014). However, in vitro experiments reveal that vitamin C has many functions (Farag *et al.,* 2018). In this sense, before being subjected to γ or X radiation, the administration of vitamin C avoids chromosomal damage in cells (Konopacka *et al.,* 1998; Mortazavi *et al.,* 2015). Many types of research show the protection of DNA by using vitamin C when it is submitted to radiation doses (Mahdi *et al.,* 2018; Yoshikawa *et al.,* 2006).

Treatment with vitamin C dramatically improved the survival of mice after total body irradiation (TBI). Thus, vitamin C is effective and efficient in preventing DNA damage in mouse tissues. (Yamamoyo *et al.,* 2010). In another study, administering vitamin C at 1, 6, 12, and 24 h after TBI improved mouse survival. The use of vitamin C was efficient in reducing cell damage by reducing free radicals (Sato *et al.,* 2015).

Thus, the administration of vitamin C before γ and X irradiation prevents chromosomal damage to cells and lethality caused by radiation. Vitamin C can prevent the adverse effects of radiation on TBI through antioxidant protection in irradiated animal tissues (Konopacka *et al.,* 1998; Mortazavi *et al.,* 2015). After ionizing radiation, DNA responds in several ways to repair the damaged structure (Mahdi *et al.,* 2018). However, the defense mechanism against radiation is not fully understood and is still a topic for discussion.

The scientific community needs answers regarding the effects of radiation and acceptable doses without harming healthy cells. The biological impact must be studied in greater depth (Hill, 2004). In this sense, this work aims to evaluate the radioprotective effect of vitamin C induced by X-rays and gamma-rays (γ-rays) in mice testis tissue.

2. MATERIALS AND METHODS:

2.1. Animals and reagents

This research was carried out at The Animal Care and Research Ethics Commission, Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) at Al-Mustansiriya University.

All requests and treatment of animals, along with the procedures intended in this study, were confirmed. Male albino Balb/c mice with eight weeks (20 ± 2 g body weight) were obtained from (ICCMGR). The mice were isolated eight days before irradiation. The mice were separated into six groups (each group with seven) placed in cages at a temperature of 22 ± 2 ºC, the relative humidity of 50 ± 10%, and a 12 hour shift of the light-dark cycle, fed standard commercial rodent chow and sterile water.

2.2. γ and X-rays procedures

The mice were placed in a specially designed and well-ventilated plastic container. In the first test, the animals received a single dose of 4 Gray (Gy) of γ-ray in TBI. This experiment used

a rate of 0.3 Gy/min with energies of 1.17 and 1.33 MeV from eight rods of ^{60}Co. The tests were carried out in the Laboratory of Nuclear Sciences at the University of Baghdad/College of Science. In the second test (same methodology above) but the mice received a single dose of 4 Gray of X-ray in TBI by a linear accelerator (Electa Compact) in the Baghdad Center for Atomic Medicine at Medical City in Baghdad/Iraq. The dose rate was 2.85 Gy/min with a distance to a source sample of 120 cm. Field size 20 × 20 cm at room temperature (23 ± 2 ºC) with 6 MeV energies. These experiments have been duplicated in both cases.

2.3. Administration of Vitamin C (Ascorbic Acid) and antibodies

Vitamin C (ALPHA CHEMIKA, India) was dissolved in physiological saline solution and administered intraperitoneally (i.p.) for 8 days at a 200 mg/kg.day dosage. Was used Anti-γ-H2AX (phospho S139) antibody [9F3] ab26350 from Abcam with 1:200 dilution.

2.4. Tissues collection and immunohistochemistry staining of γH2AX in testis tissues

The mice were sacrificed after 1, 3, and 24 hours of irradiation with the collection of testicular tissues. The tissues were placed in formalin solution, neutral buffered, 10% for 24 hours, transferred to 70% ethanol, and embedded in paraffin. The development of this internship was carried out at the ICCMGR.

Immunohistochemistry (IHC) was performed in 5 μm sections, using the following antibodies: γ-H2AX (phospho S139) antibody [9F3] ab26350 from Abcam with 1: 200 dilution.

An optical microscope with a CCD camera (OLYMPUS OPTICAL, Model CX4IRF) was used to detect the foci with 40x magnification. The images were obtained in the Unit of Medical Physics, Department of Physiology, College of Medicine / Al Mustansiriyah University, Baghdad, Iraq. The foci were divided into four tissue sites, with each site occupying 100 cells. Each cell is classified according to the number of foci in the DSB. Then, an average of the four sites was calculated, and the final result of preparing the foci was divided into mild (0-3), moderate (4-6), severe (≥ 7).

2.5. Statistical evaluation

The data were analyzed using SPSS statistical software. A t-test was used to determine the difference between the non-irradiated sample (control) and the irradiated samples. A P-value was determined based on the difference between the non-irradiated sample and the irradiated samples. Based on these results, $P < 0.05$ is considered significant.

3. RESULTS AND DISCUSSION:

3.1. DSB detection by γ-H2AX foci on irradiated mice testis tissues with γ-ray pre and post-treatment with vitamin C

As the dose of gamma radiation interacted with the sample with vitamin C and without vitamin C, the number of foci was changed. The results are shown in Figure 1. The levels of γ-H2AX in irradiated (γ-ray) and non-irradiated samples using vitamin C did not differ significantly ($P > 0.05$).

In 1h, the foci groups (0-3), (4-6) did not show significant differences ($P > 0.05$) between irradiated and non-irradiated vitamin C. However, for the foci group (≥ 7), there was a decrease ($P < 0.01$) between non-irradiated samples (control). For 3 hours, the foci groups (0-3) and (≥ 7) showed a significant increase. Therefore, for the foci group (4-6), there was no important difference ($P > 0.05$). In turn, within 24 h, the focus groups (0-3), (4-6), and (≥ 7) do not show any significant difference ($P > 0.05$).

Figure 2 shows the endogenous damage to DNA and DSB in testicular tissues of mice (with and without vitamin C) by γ-rays. It was identified some differences in the microscopic images in the irradiated and non-irradiated samples. As the time of exposure to γ-rays increases, it was noticed a decrease in the focus groups. This behavior is not evident for the focus groups that used vitamin C.

3.2. DSB detection by γ H2AX foci on irradiated mice testis tissues with X-ray pre and post-treatment with vitamin C

Figure 3 shows the effects of X-rays on vitamin C. It was found significant differences when the testicular tissues of mice are exposed to X-rays without vitamin C (Figure 4). Thus, there was an increase in the number of foci for samples not treated with vitamin C.

Figure 3 shows the foci levels, taking into account the use or not of vitamin C combined with the time of exposure to X-ray. The results allowed us to conclude that there were no significant changes ($P > 0.05$) in the focus groups (0-3), (4-6)

with the use of vitamin C without radiation and for 1 and 3 h under the influence of X. For the focus group ≥ 7, there were differences (P ≤ 0.01) in the 1 and 3 h radiation. In general, in 24 hours for all focus groups, there was no significant change.

Figure 4 shows the damage to endogenous DNA and DSB in testicular tissues of mice that received doses of X-rays with and without vitamin C. In the times of 3 and 24 hours, the focus groups showed low levels using vitamin C. Significant variations occurred in 1 h. The decline rate is more evident in the groups that used vitamin C, taking into account the formation of foci of the DSB. These observations indicate that treatment with vitamin C before irradiation had positive results in reducing DSB. Thus, the decrease in the number of foci in an γ-H2AX indicator.

3.3. Comparison between foci in γ and X-rays (control and vitamin C groups) to detect DSB in DNA by γ-H2AX

To evaluate the effects of ionizing radiation (γ and X-rays) in testicular tissues of rats, vitamin C, and without vitamin (control) were used. Figures 5 and 7 reveal that the focus groups (0-3), (4-6), (≥ 7) showed no significant difference (P > 0.05). Figures 6 and 8 show us the images of the IHC with the γ-H2AX antibody at different times (without irradiation, 1 h, 3 h, 24 h). Microscopic images evidenced endogenous damage to DNA and DSB in tissues irradiated with γ-X rays with and without vitamin C. These results indicate that there is no apparent difference between the groups (γ, X-rays) with and without vitamin C concerning the γ-H2AX foci in the DNA.

Tissues and organs specific from individuals vary significantly when subjected to radiation. Growing male germ cells are more susceptible to disturbances and are recognized as a radiosensitive organ. (Lakshmi and Kesavan, 1993). The damaging effects of ionizing radiation on the mice testis tissues are well documented (Paull *et al.,* 2000).

This study shows that the radioprotective agent (vitamin C) acted effectively when the samples were subjected to X-rays and γ (Taş *et al.,* 2014). In addition, it decreased DNA damage in testicular tissues and the preservation of genetic material (Marjault and Allemand, 2016).

When evaluating DSBs after exposure of 4 Gy (γ-rays) for the group that administered vitamin C, tissue regeneration levels had a progressive decrease (Fig 1 and 2). However, the results were inconsistent for the control group (without vitamin C), as there was no protection against radiation. Thus, it was concluded that the protective effect is accompanied by doses of vitamin C, as evidenced by several research groups today (Brand *et al.,* 2015; Mortazavi *et al.,* 2015; Paull *et al.,* 2000; Sato *et al.,* 2015; Sukhotnik *et al.,* 2018).

The ionizing radiation that interacted in the DSBs correlated significantly with the dose of X-rays and γ (Hill, 2004). The use of the antibody (γ-H2AX), as a marker for DSBs, was found in approximately a few minutes after irradiation (Mahdi *et al.,* 2018). The increase in DSBs, followed by a reduction, represented the repair of DSBs (Hamer *et al.,* 2003). Therefore, the number of γ-H2AX foci decreases rapidly over time (Rothkamm and Horn, 2009). This is due to the histone H2A protein presence, which acted on the DSBs and on the DNA (Jeggo and Löbrich, 2006). Figures 3 and 4 showed a similar result in Figures 1 and 2 in the amount of γ-H2AX foci, irradiated by X-rays in 1, 3, 24 h. DSBs are caused by DNA damage caused by ionizing radiation (Löbrich *et al.,* 2005; Rothkamm and Löbrich, 2003).

Imaging the foci is essential to check the effect of radiation inside cells representing DSB DNAs (Rogakou *et al.,* 1998). Previous studies have shown that damage to DSBs is correlated with X-ray and γ doses (Kuefner *et al.,* 2010). There is an increase in DSB levels between 30 and 60 minutes after radiation, followed by repairs to the DSB (Löbrich *et al.,* 2005). Therefore, studies confirm the effective role of vitamin C in reducing the risk caused by ionizing radiation. In this sense, the results obtained in this study are in line with the research carried out by several groups addressed in this work.

In this study, the effect of X-rays and γ in mouse testis, using a dose of 4 Gy, had the same effect on DNA damage with a slight difference in the proportion of foci formed due to DSBs Figures 5 – 8. This indicates that only one type of ionizing radiation will be sufficient to carry out experiments in vivo. Previous studies have proven that the use of 2.2 Gy of X-rays in the colon tissue in mice produces desirable effects. However, on biological tissues, a different source and energy will depend on the doses used (Graupner *et al.,* 2017).

Thus, the effective role of vitamin C in preserving testicular tissue, submitted to X-rays and γ, is evident. The probable cause of the decrease in the number of foci formed after several hours of radiation can be explained by the low production of free radicals in the DNA. Vitamin C takes some time to enter the cell's nucleus to

intercept free radicals produced near DNA. Simultaneously, the amount of γ-H2AX foci, after exposure to 4 Gy, increased without the administration of vitamin C in the control group. The use of vitamin C in a mouse had a strong inhibitory effect, which prevented damage to testicular tissues and prevented post-irradiation fertility loss.

4. CONCLUSIONS:

In view of the results obtained in this research, it was concluded that the testicular tissues of mice exposed to ionizing radiation (γ or X) suffered DNA damage. However, as a radioprotective agent, the use of vitamin C reduced the effects on the genetic code. The effect of X-rays and γ on the testicles of mice, at doses of 4 Gy, had the same effect on DNA. In this way, the use of ionizing radiation can be safe *in vivo* experiments. Although the source of energy used (photons) is different, the biological effects are similar.

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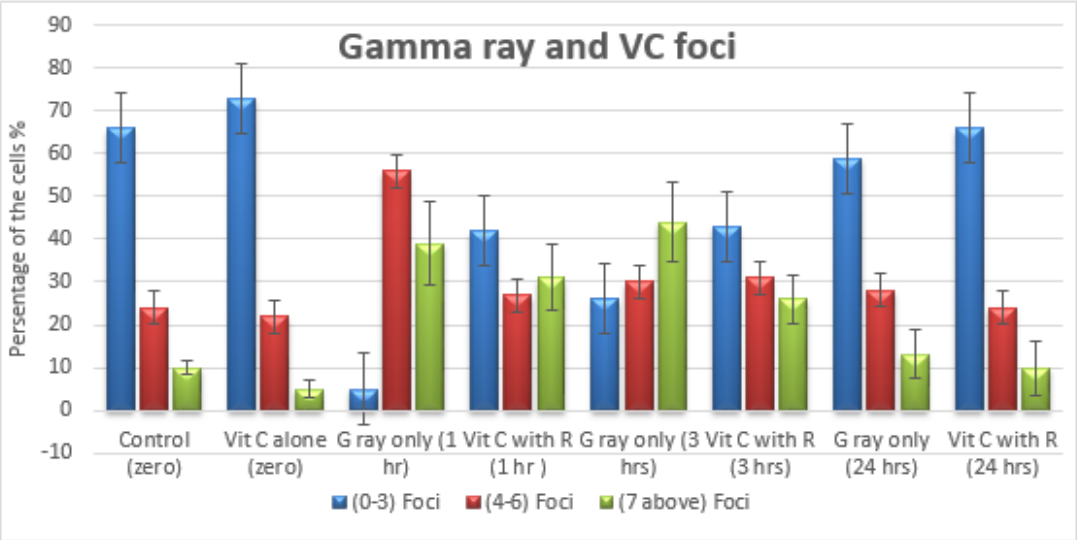

Figure 1. *DSB and γ-H2AX foci in control and VC with and without IR (γ-ray) at different times.*

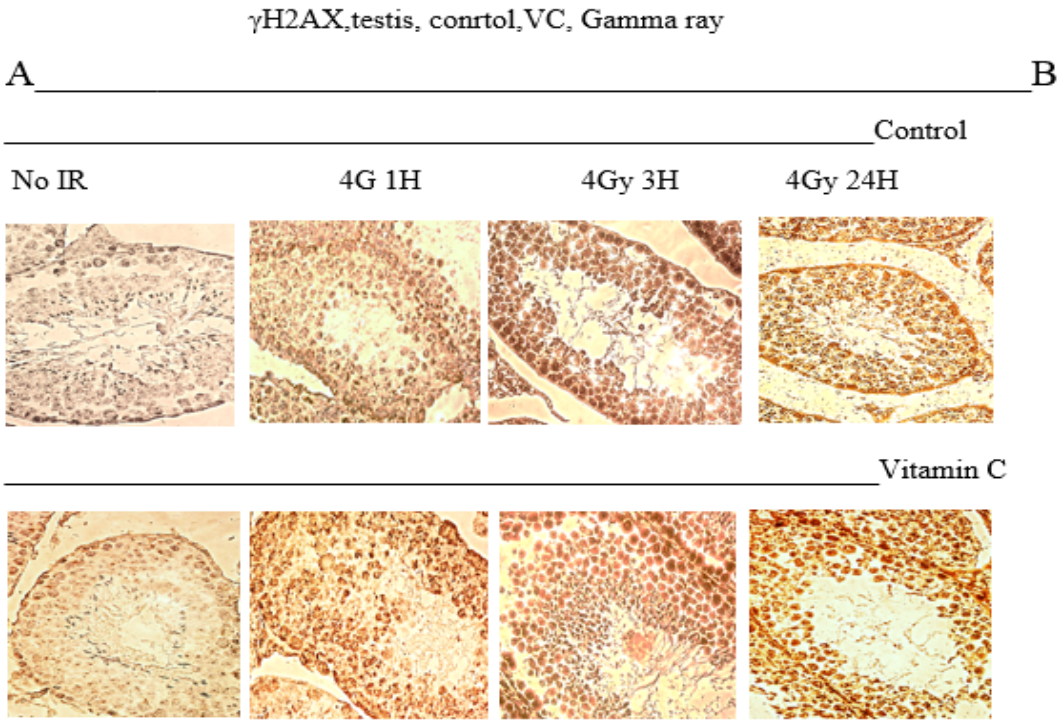

Figure 2. *Endogenous DNA damage and DSB capability in control and VC groups with and without IR (Gamma-ray). A and B, Representative IHC images (A) and quantification (B) of γ-H2AX foci formation in testis tissue before and after (4 Gy) of IR in (1, 3, 24 h).*

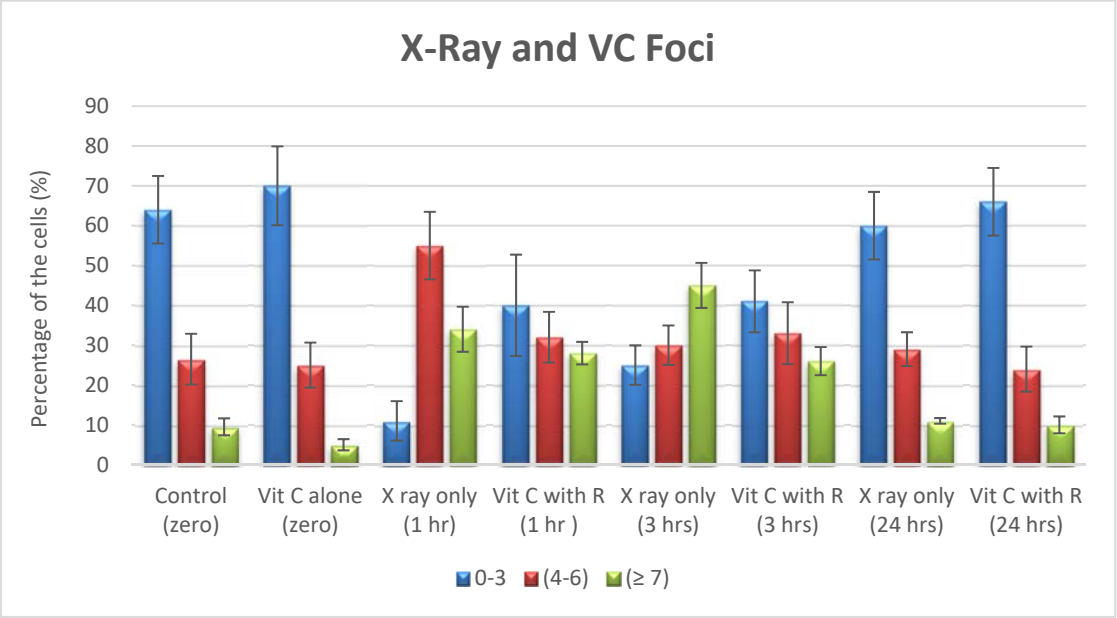

Figure 3. *DSB and γ-H2AX foci in control and VC with and without IR (X-ray) in different times.*

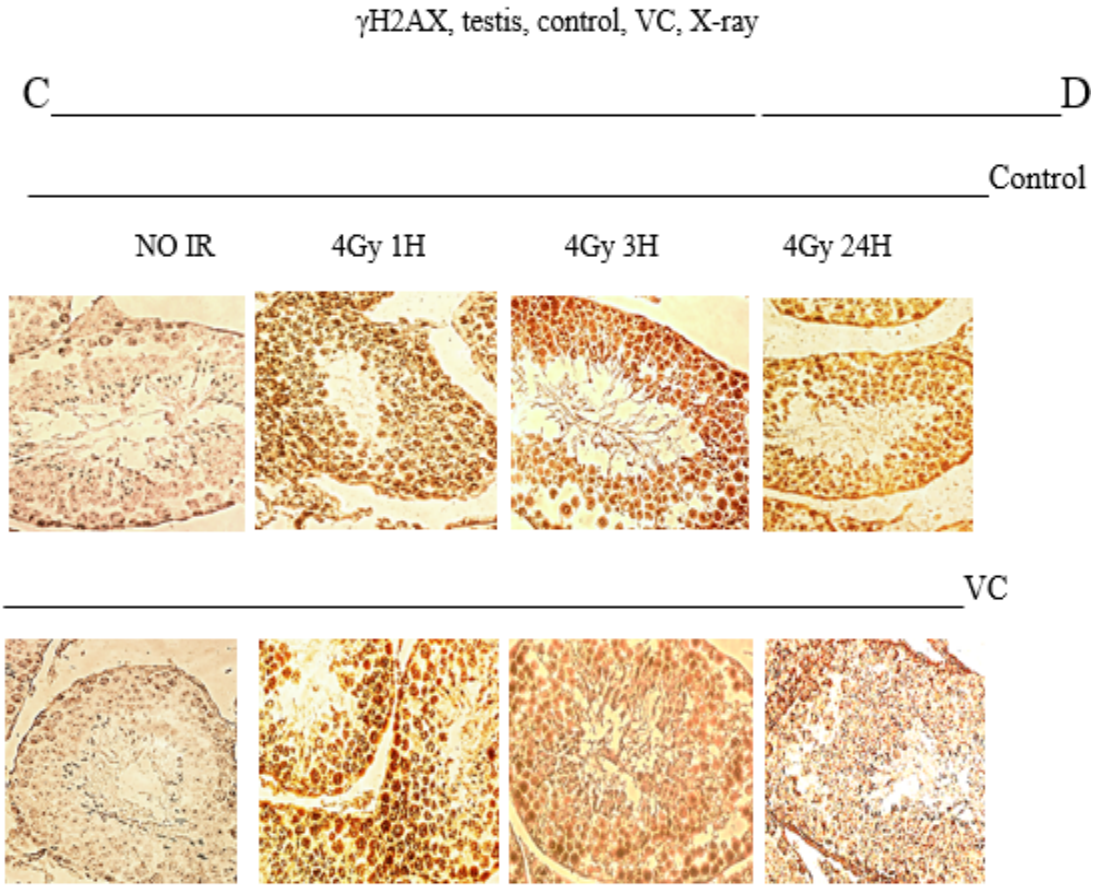

Figure 4. *Endogenous DNA damage and DSB capability in control and VC groups with and without IR (X-ray). C and D, Representative IHC images (C) and quantification (D) of γ-H2AX foci formation in testis tissue before and after (4 Gy) of IR in (1, 3, 24 h).*

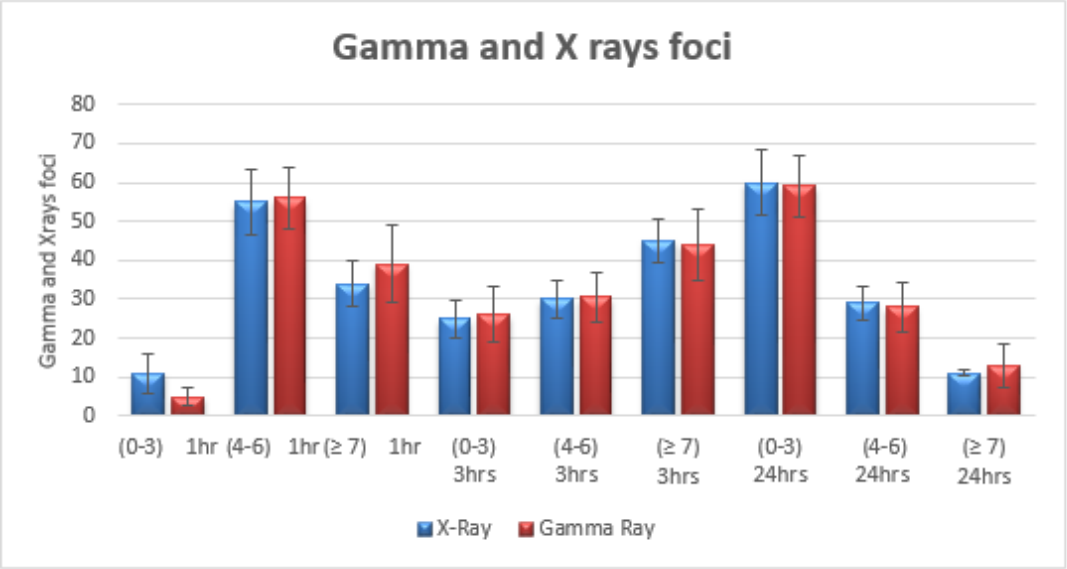

Figure 5. *Comparison between Gamma and X-rays in the control group represents γ-H2AX foci of DSB in DNA.*

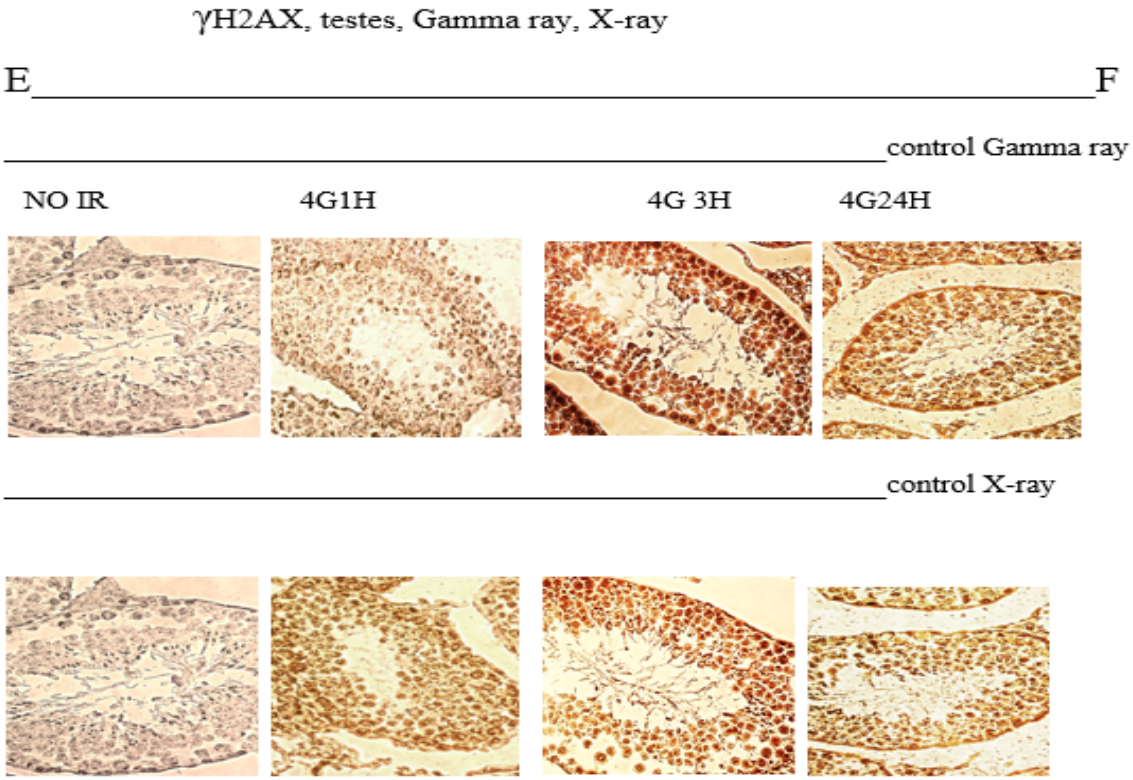

Figure 6. *DNA damage and DSB capability in control groups of (γ and X-rays). E and F, Representative IHC images (E) and quantification (F) of γ-H2AX foci formation in testis tissue before and after (4 Gy) of IR in (1, 3, 24 h).*

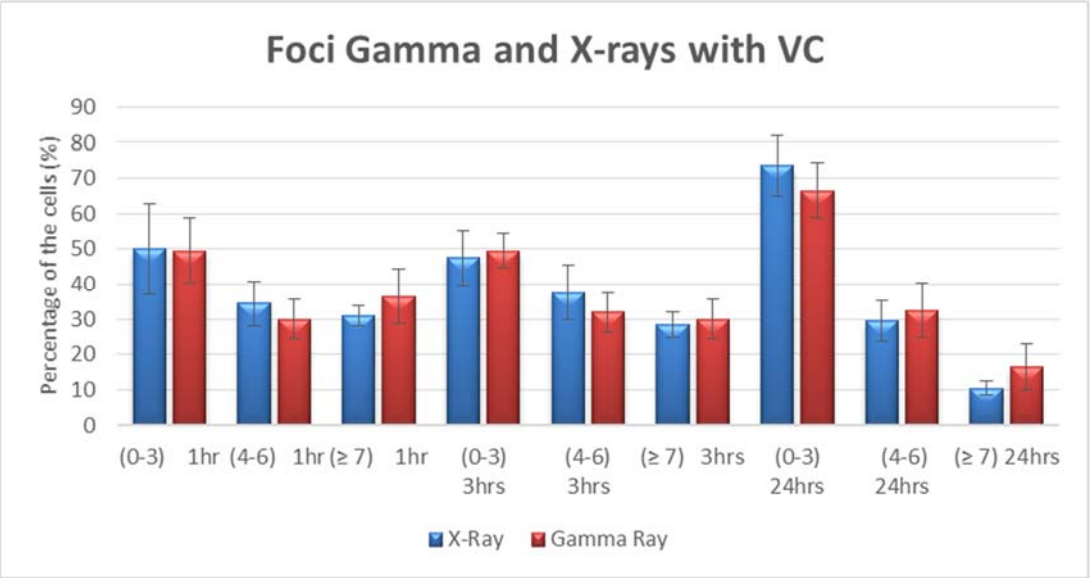

Figure 7. *Comparison between Gamma and X-rays administrated with VC represent γ-H2AX foci of DSB in DNA.*

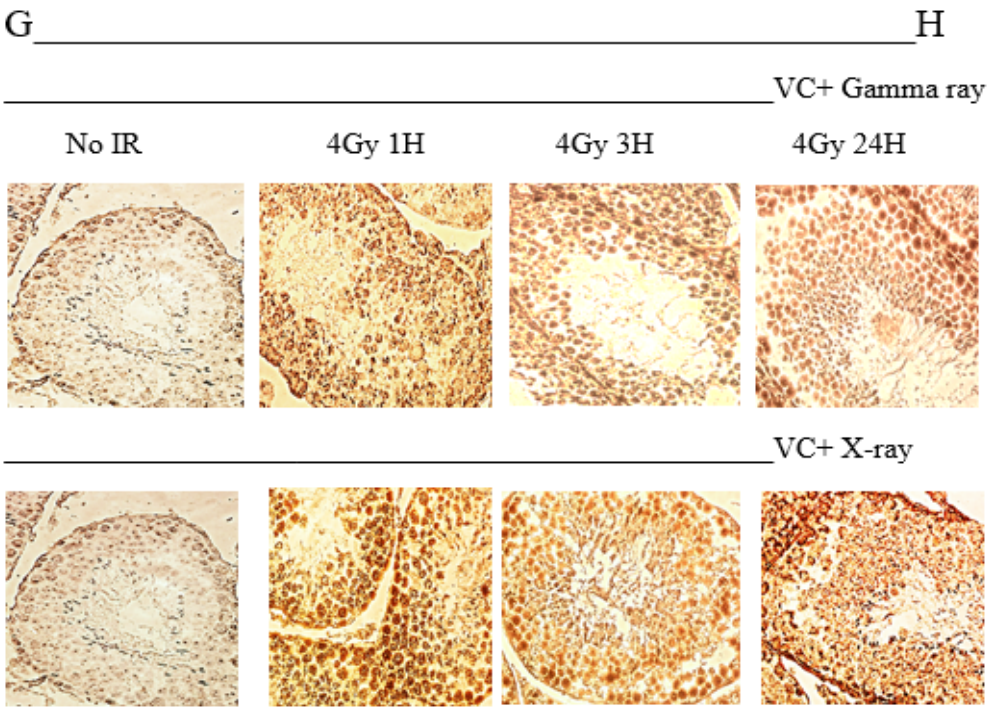

Figure 8. *DNA damage and DSB capability in VC groups of (γ and X-rays). G and H, Representative IHC images (G) and quantification (H) of γ-H2AX foci formation in testis tissue before and after (4 Gy) of IR in (1, 3, 24 h).*