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THE PROBABLE RADIOPROTECTIVE ROLE OF *Acacia nilotica L.* AGAINST BIOCHEMICAL AND CYTOGENETIC DISORDERS INDUCED IN GAMMA IRRADIATED MALE RATS

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Abstract

Gum Arabic (*Acacia nilotica L*.) is a respected plant that has many nutrients and curative practices. It hinders, improves, or manages many disorders. The radio-protective activity of *Acacia nilotica* was investigated against γ-rays-induced testicle damage in rats. Twenty-four rats were correspondingly distributed into 4 groups; control, *Acacia nilotica* (15mg/kg, daily for 30 days), γ-irradiated (5Gy γ-rays, single dose) and *Acacia nilotica* plus γ-rays treated groups. The plasma testosterone and total antioxidant status (TAS) were estimated. Lipid peroxidation; malondialdehyde (MDA), reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), also glutathione peroxidase (GPx), catalase (CAT), tumor necrosis factor-α (TNF-α) with interleukin-1β (IL-1β), were determined in the testicle tissues. A testis weight, sperm count and motility, peripheral-blood and bone-marrow micronuclei (PMN and BMN), and frequency of chromosomal aberrations (CAs) were scored. A significant decline in the levels of plasma testosterone with TAS observed in the γ-irradiated rats. The results also showed significantly increased levels of testicle MDA, inflammatory markers, PMN, BMN and CAs frequencies and decrease in testes weight, sperm count and motility and levels of testicle antioxidants markers in gamma irradiated group. All these biochemical and fertility indices results were significantly enhanced in the *Acacia nilotica* plus γ-rays treated groups. However, the possible alleviate activity of *Acacia nilotica* on γ-rays-induced testicle injury in rats has not previously conversed, and this is the topic of this study.

Keywords: *Acacia nilotica*. Genetics. Histopathology. Radioprotector. Rats. Testis. γ-rays.

1. Introduction

Gamma radiation-induced oxidative damage in all rats' organs, it produces reactive oxygen species (ROS) that lead to lessen the tissue antioxidant defense system (Hassan et al. 2021) and involves in the progression of cellular macro- and micro-molecules, proteins, DNA damages, and mutagenesis (Inoue et al. 2020).Whole body γ-irradiated (5-10 Gy)-induced testicular damage, sperm abnormalities and/or impair spermatogenesis in rats (Gawishet al. 2020; Said et al. 2020). In a recent study, the authors decided that gamma-rays stimulated a substantial alteration of testicular biochemical indices: a decline in the level of serum testosterone, besides a decreased level of testicular reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase. Also, it induced an increased level of

malondialdehyde, nitric oxide and the level of the inflammatory markers in the testis of gamma-irradiated group, as well as augmented the sperm abnormalities percentage (Gawishet al. 2020; Said et al. 2020). In an attempt to lessening these cytotoxicity properties, antioxidant bioactive compounds been recognized to deactivate radiation-concomitant toxicities (Mahgoub et al. 2020; Sah et al. 2021).

Acacia species seeds are one of the richest sources of food for individuals especially for the in borne Australian people (Adiamo et al. 2021). It has been used for the treatment of tumor (Malami et al. 2020) in addition to treating dysentery and tuberculosis (Hernández-García et al. 2019). Furthermore, the *Acacia* species have various biological activities include immunomodulatory (Kaddam et al. 2020), wound healing (Moglad et al. 2020), chemo-preventive (Yada et al. 2020), vasoconstrictor (Mohammad et al. 2018),antifungal (Salem et al. 2021), antiplatelet aggregation (Saluet al. 2019) and anti-hepatitis (Siddiquiet al. 2017). There are many beneficial properties of *Acacianilotica* comprising antimicrobial, antiparasitic, antidiarrheal, antihyperlipidemic, antihypertensive, antispasmodic, antipyretic, antinociceptive, antiulcer, antispasmodic, antidiabetic, anticancer, antimutagenic, anti-inflammatory, and antioxidant activities (Abduljawad 2020). Besides, the seeds of *Acacia nilotica* have been used as a flavoring agent in dietary and beverages products (Apolinar-Valiente et al. 2019).

Several biologically active compounds comprise flavonoids, phenolics, tannins, terpenoids, saponins, alkaloids, and polysaccharides including bioactive agents such as kaempferol, isoquercitin, apigenin, umbelliferone, rutin, naringenin, lupane, gallic acid,ellagic acid, niloticane, catechin, β-sitoterol and androstene were isolated from different parts of *Acacia* species (Ayeet al. 2019; Casas et al. 2019).Various studies evaluate the constructive special effects of *Acacia nilotica* upon oxidative burden-induced testicular damages in rat (Al-Doaiss and Al-Shehri, 2020). It was recommended that *Acacia nilotica* might have a protecting role against oxidative stress-induced compromised testicle functions in diabetic rats (Al-Doaiss and Al-Shehri, 2020). We have endeavored to appraise the medicinal and biological role of *Acacia nilotica*, as an accepted remedy for moderation of gamma-rays-induced testicular damage. Biochemical and genotoxic disorders measures in serum and testicles of rats were performed.

2. Material and Methods

Animals

Male albino rats (280–300 g) were supplied from the animal house of Egyptian organization for biological product and vaccines Giza, Egypt. Rats were kept in cages and acclimatized to the laboratory situations for a week before to the experiment and were supplied with proper access to rat diet and water *ad libitum* and maintained under average environmental conditions of humidity, temperature (20-22°C), and 12-hours light/dark period. Animals were destitute of food, but not water, overnight at the end of the experiments. All experimental animals' procedures performed according to the approval of the Research Ethics Committee (REC- NCRRT), protocol number: 23A/20, according to the guidelines of the care and use of laboratory animals (NRC 2011).

Radiation processing

It was done using of gamma cell-40 (cesium-137) found at NCRRT, Cairo, Egypt. Rats were irradiated with a whole body single dose level of 5 Gy γ-rays, delivered at a dose rate of 0.4 Gy/ min at the time of the conducting tests. This dose is adequate to prompt testicular damage or toxicity in rats and it was recognized that it is a repairable dosage (Gawish et al. 2020). Besides, it presents the sublethal dosage for rats that agrees with the study of Rosen et al. (2020).

Chemicals

Pure medicinal grade, *Acacia nilotica*seeds powder extracts (ASIN: B07SJP7PGF) were purchased from VINARGHYA Pharmaceuticals, Maharashtra, India. All other chemicals and solvents used were of the highest research rank from Sigma-Aldrich, USA. Radioimmunoassay kit (No. TKTT1, Los Angeles, USA and

BioSources international (Camarillo, USA) and the total antioxidant status (TAS) using colorimetric Randox laboratories kit, UK.

Experimental design

Animal grouping

Rats were casually spread into four groups of 6 rats. The control group: received 2 ml sterile water (S/W)/ day for 30 days, intragastric (ig). *Acacia nilotica* group: received *Acacia nilotica* dissolved in 2 ml S/W just before use (15 mg/ kg body weight, ig) for the same interval, according to Al-Doaiss and Al-Shehri, (2020). Gamm-irradiated group: received 2 ml S/W with the same dose and period; then exposed to a single dose of (5Gy γ-rays). *Acacia nilotica* + γ-rays group: received *Acacia nilotica* with the same dosage and interval just before irradiation. The animals were dislocated (cervical dislocation) and their testes were separated.

Sampling

The blood samples were collected by heart pinhole and plasma was separated under the usual laboratory settings. The right Coda epididymis and testes organs were washed with ice-cold saline and then testes were weighed and dichotomized for biochemical studies. Blood plasma was prepared for assessing the testosterone level using ELIZA kits.

Biochemical investigation

The testicles tissues were cut into minor slices and were homogenized into 50mM, 1:10 Triose/HCl buffer, w/v (pH7.4) using Lab-homogenizer, MNW-302, Poland for two minutes then the homogenates were centrifuged at 3000xg for eight minutes to get clear supernatant. The testis homogenate samples were stored at −20°C for oxidative stress analysis. In the testicle tissue samples, levels of malondialdehyde (MDA) and reduced glutathione (GSH) as well as activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes were assessed using available commercial kits (Zellbio-GmbH,Baden-Württemberg, Germany) according to the company's procedures. The absorbance was read at 412, 412, 535 and 405, 420 nm, respectively.

The total protein level in testicle tissue was evaluated using the total protein kit (AMP-diagnostics, Austria) depending on the bovine serum albumin as a standard. Revealing of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the testicle's homogenate were implemented by ELISA kit group, BioSources International (Camarillo, USA), belonging to the corporation's instructions. Each test measure was repeated 3 times.

Cytogenetic analysis

Peripheral blood micronucleus (PMN) assay: Peripheral blood-smears were arranged as designated early (Holden et al. 1997). Few blood drops were obtained from tail tip of rats; the blood smears were ready on cleaned glass slides, allowed to dry at 37°C then fixed into absolute methanol alcohol for 3 minutes. Afterward fixation, the slides were stained with acridine orange and washed twice into phosphate buffer, pH 6.8 (Hayashi et al. 1994). The bone marrow micronucleus (BMN) assessment was prepared as Bhunya and Jena (1996) described early: Each rat's femur bone marrow cells were flushed out, collected in centrifuge tubes, and washed into 2 ml fetal calf serum (Sigma-Aldrich, USA). The cell suspension was homogenized and centrifuged at 1000xg for 10 minutes, and then the supernatant was castoff leaving few drops of fetal calf serum. The cell pellet was re-suspended in the residual drops of the fetal calf serum and then smeared on clean, dry glass slides. The slides were fixed with absolute methanol alcohol for 3 minutes then, stained for 5 minutes into 5% Giemsa stain diluted into phosphate buffer, v/v, pH 6.8 (Sigma-Aldrich, USA). The slides were recorded according to the established criteria of Titenko-Holland et al. (1997).

Frequency of chromosomal aberrations (CAs): The method in detail: Two hours before rats' cervical dislocations, each rat was injected with a single dose of 0.2ml colchicine (1%) via intraperitoneal. One femur was dissected after dislocation then, the bone marrow cells were flushed out into 2ml of 0.9% NaCl solution and centrifuged. The supernatant bone marrow-suspension was washed into chilled 0.075M KCl hypotonic solution and fixed into 1:3, v/v acetic acid and methanol solutions. The chromosome plates were air-dried then stained into 5% Giemsa-phosphate buffer (pH 6.8). Fifty metaphases were scored for each rat and aberrations types were categorized in accordance with Albertini et al. (2000).

Fertility metrics

The right cauda epididymis was regular into a Petri-dish with 10 ml phosphate-buffered saline (PBS), 0.1 M, pH7.4 for sperm count and motility estimate. Sperm analysts based on the method formerly reported in Sahin et al. (2016) report. Sperm count was prepared using a glass hemocytometer under an ordinary light optical microscope. A special glass cover slide was placed onto the hemocytometer slide then, a drop of 10µl of caudal epididymis sperm suspension was put down below the cover slide. Also, the revealed hemocytometer was processed for sperm motility interpretation; a glass cover slide was located on the hemocytometer then, a drop with 10µl of the prepared caudal epididymis sperm suspension was placed below the cover slide.

Statistical analysis

Data were analyzed using ANOVA (one-way analysis of variance) followed by LSD-post hoc pattern. The results obtained were expressed by mean± standard error (S. E.). Differences were considered significant at $p \leq 0.05$ (Snedecor and Cochran 1994).

3. Results

There were no significant differences observed between the values of all biochemical indices in plasma or in testicle tissues in *Acacia nilotica* group as compared with that of the control group.

The variations in blood plasma testosterone concentration and TAS activity in male rats are given in Figures 1 and 2. The γ-irradiated group revealed significant decreases ($p< 0.05$) in plasma testosterone level and TAS-activity as compared to the control and *Acacia nilotica* groups.

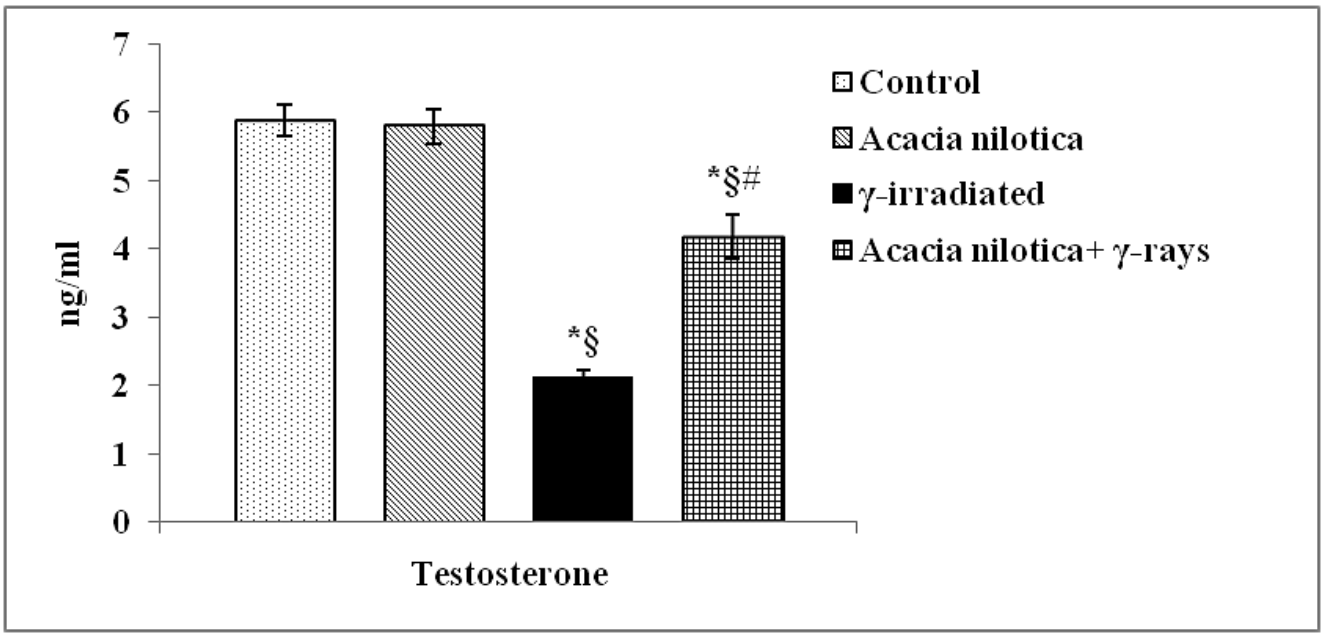

Figure 1. Influence of *Acacia nilotica* and/or γ-rays on testosterone concentration in plasma of different rat groups. *$P< 0.05$ as compared to control group; §$P< 0.05$ as compared to *Acacia nilotica* group; #$P< 0.05$ as compared to γ-irradiated group.

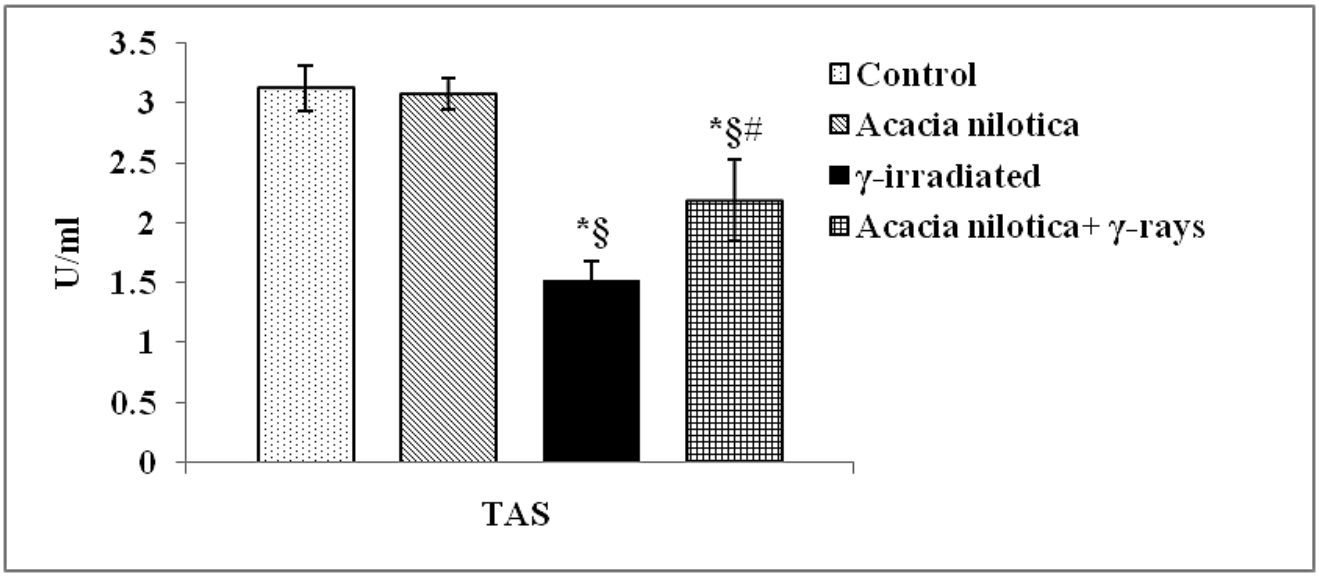

Figure 2. Influence of *Acacia nilotica* and/or γ-rays on TAS activity in plasma of different rat groups. TAS= Total antioxidant status; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

Significant increases (p< 0.05) in testosterone level and TAS-activity were detected in *Acacia nilotica*+ γ-rays treated group as compared to γ-irradiated group but there were still significant differences when compared to control and *Acacia nilotica* groups in Figures 1 and 2.

The values of MDA and GSH concentrations in the γ-irradiated group were altered significantly (p< 0.05) as compared with that of the control and *Acacia nilotica* groups (Figures 3 and 4). On the other hand, the MDA value was significantly decreased and the GSH value was significantly increased in the *Acacia nilotica*+ γ-rays treated group in comparison with that of the γ-irradiated group but there was still significant difference (p< 0.05) when compared to control and *Acacia nilotica* groups. For SOD, CAT and GPx values were significantly decreased in the γ-irradiated group as compared with the control *Acacia nilotica* groups (Figures 5 and 6). But these values increased significantly in the *Acacia nilotica*+ γ-rays treated group when compared with the γ-irradiated group but there was a still significant difference (p< 0.05) when compared to control and *Acacia nilotica* groups.

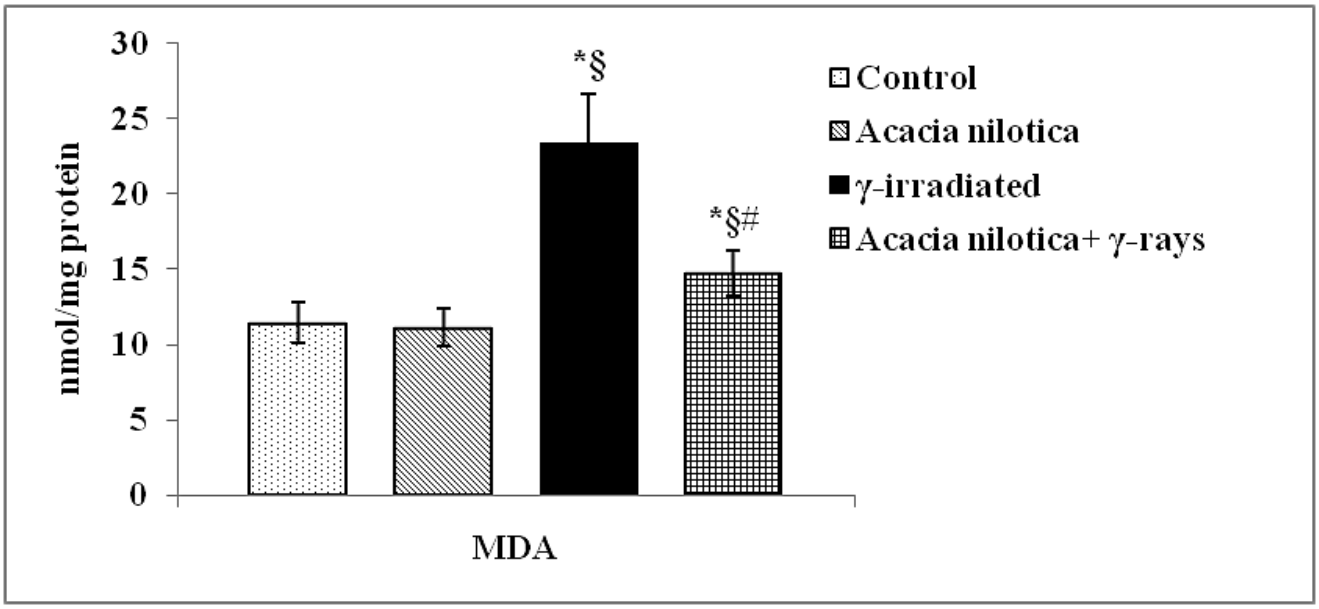

Figure 3. Influence of *Acacia nilotica* and/or γ-rays on content of MDA in testicle tissue of different rat groups. MDA= Malondialdehyde; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

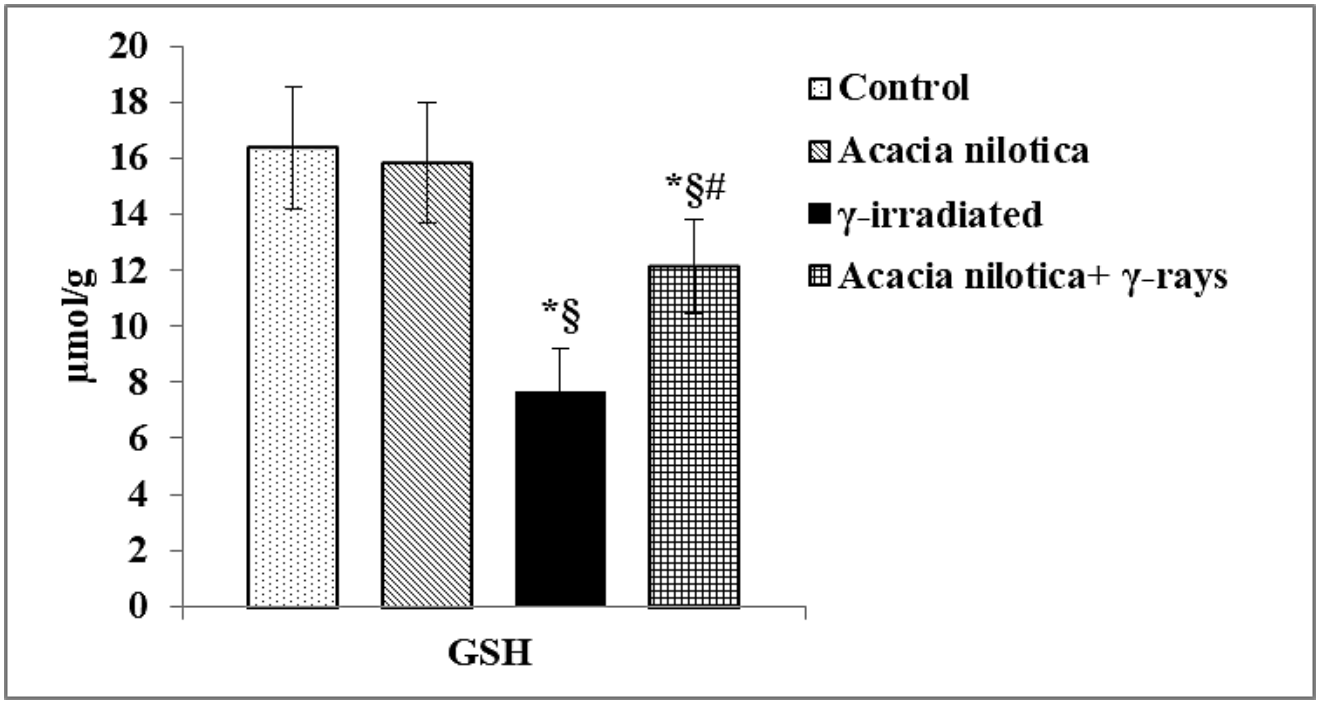

Figure 4. Influence of *Acacia nilotica* and/or γ-rays on content of GSH in testicle tissue of different rat groups. GSH= Reduced glutathione; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

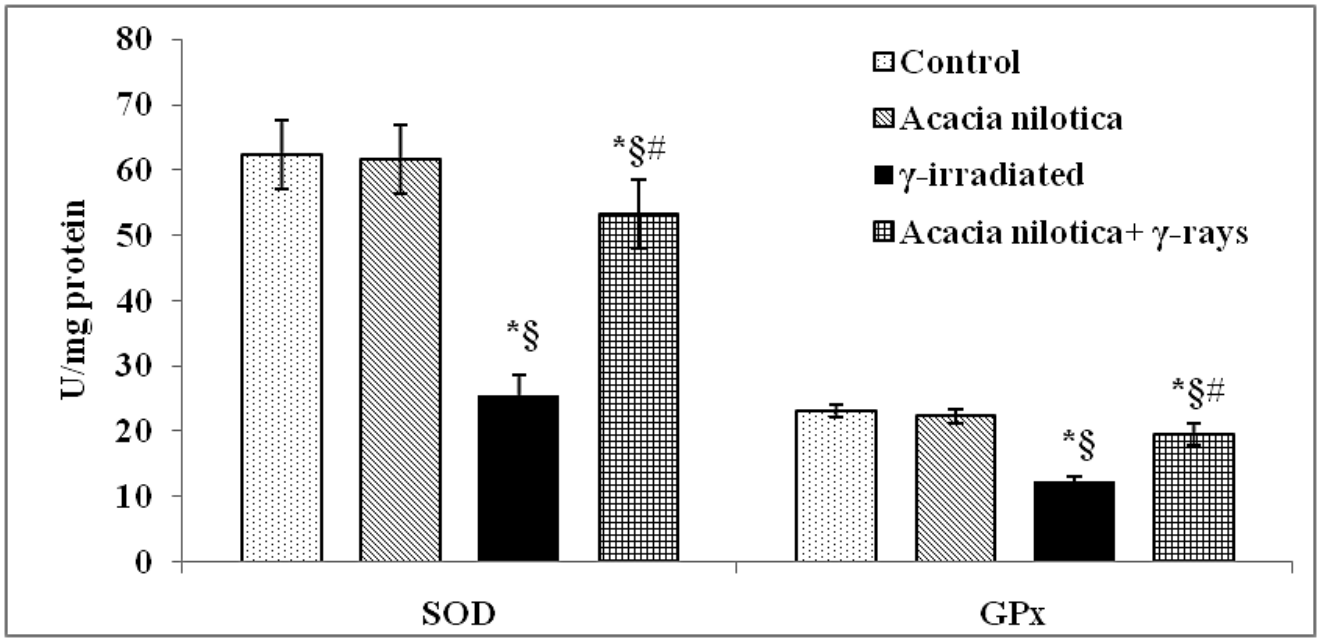

Figure 5. Influence of Acacia nilotica and/or γ-rays on activity of SOD and GPx in testicle tissue of different rat groups. SOD= Superoxide dismutase; GPx= Glutathione peroxidase; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

Figures 7 and 8 shows that γ-rays-exposure significantly (p< 0.05) elevated the concentrations of TNF-α and IL-1β, when compared to control and *Acacia nilotica* groups. Concomitant treatment with *Acacia nilotica*+ γ-rays significantly ameliorated the raises in the concentrations of these inflammatory markers (p< 0.05) when compared with γ-irradiated group but there was still significant difference (p< 0.05) when compared to control and *Acacia nilotica* groups.

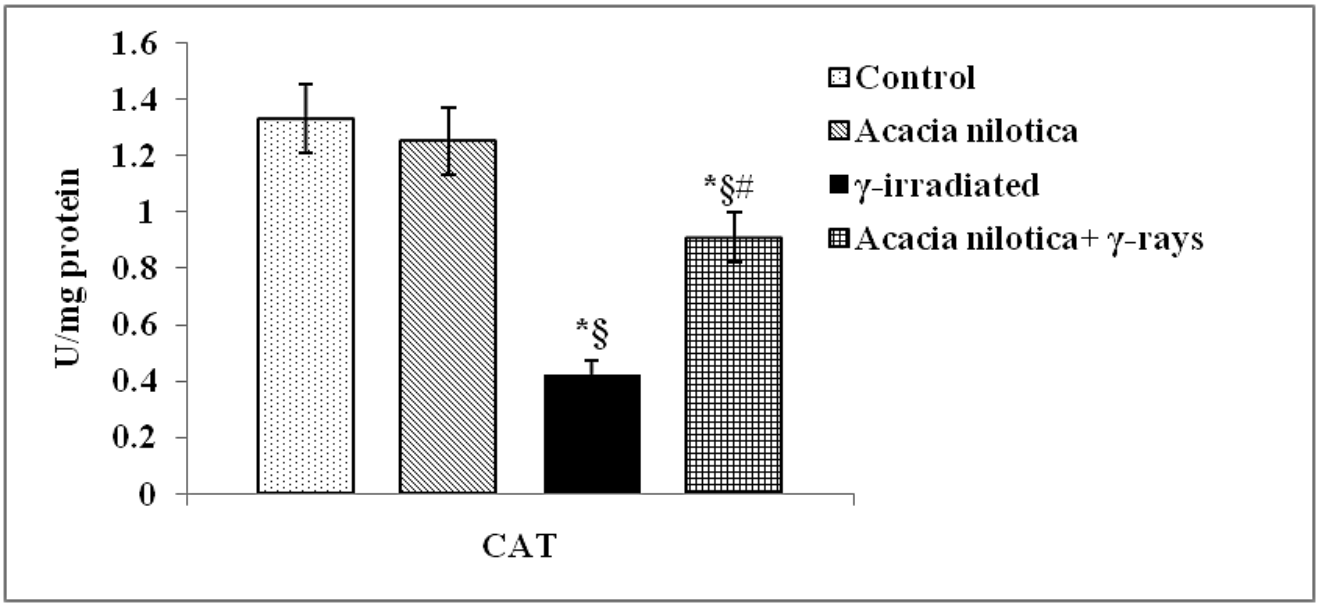

Figure 6. Influence of Acacia nilotica and/or γ-rays on activity of CAT in testicle tissue of different rat groups. CAT= Catalase; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

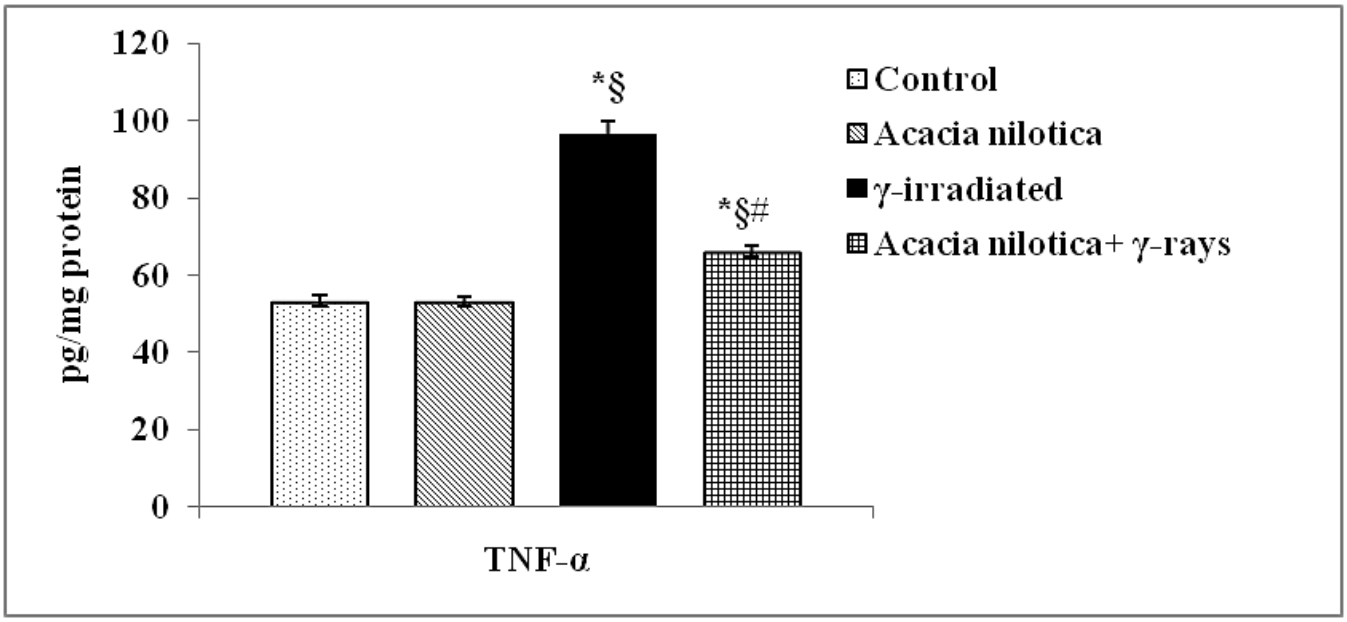

Figure 7. Influence of Acacia nilotica and/or γ-rays on level of TNF-α in testicle tissue of different rat groups. TNF-α= Tumor necrosis factor-α;*P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

After, 30 days post γ-irradiation, the rats presented a statistically significant (p< 0.05) reduction in their right testes weight, sperm count and total sperm motility percentage as compared to control and *Acacia nilotica* groups (Table 1). Treatment with *Acacia nilotica* significantly improved the right testes weight and showed a statistically significant (p< 0.05) increases in the two sperm characteristics; sperm count and percentage of total sperm motility as compared to the γ-irradiated group but there was still a significant difference (p< 0.05) when compared to control and *Acacia nilotica* groups.

High frequencies of PMN (↑ 336 and 322 %) and BMN (↑ 695 and 615 %) were noticed in the γ-irradiated group when compared to control and *Acacia nilotica* groups, respectively. However, the administration of *Acacia nilotica* pre-exposure to γ-irradiation provided a significant decline in the cytogenetic impairment when compared to γ-irradiated group reached (↓44 and ↓47 %), respectively but there was still significant difference when compared to control and *Acacia nilotica* groups (Figure 9).

A significant increment in the number of abnormal cell plates was detected in γ-irradiated group (↑900 %) when compared to control or *Acacia* group. When *Acacia nilotica* was applied, the augmented

number of chromosome aberrations was reduced significantly in *Acacia nilotica*+ γ-rays group but there was still significant difference when compared to control and *Acacia nilotica* groups (↓50 %) in Table 2.

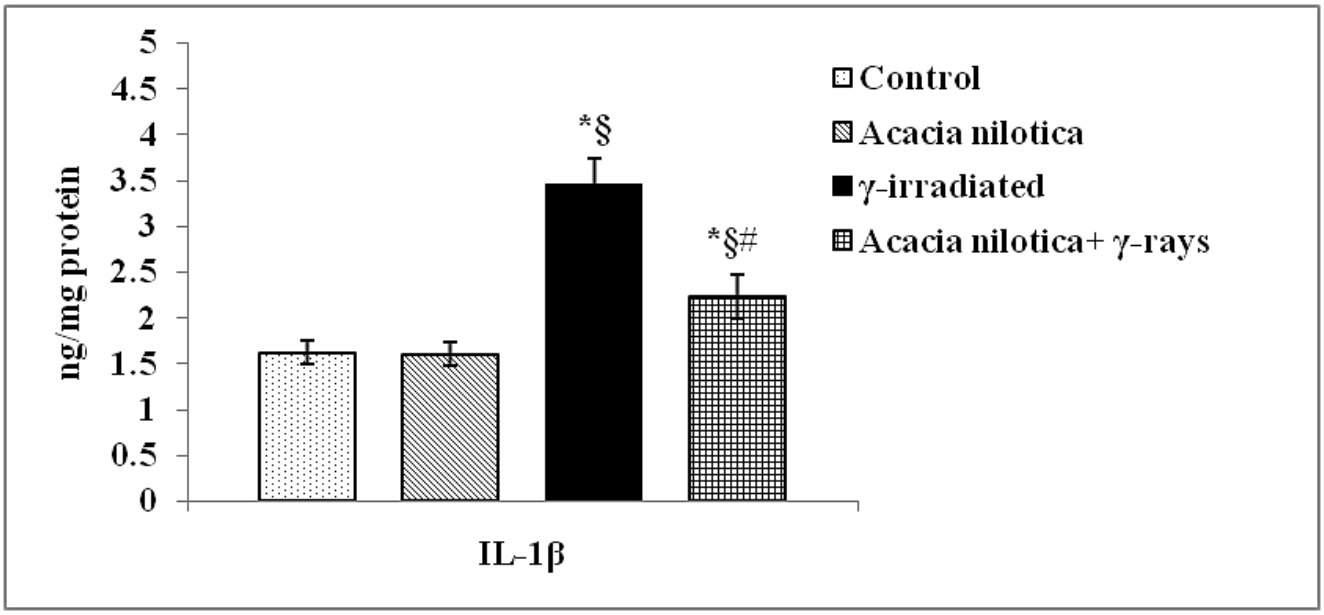

Figure 8. Influence of Acacia nilotica and/or γ-rays on level of IL-1β in testicle tissue of different rat groups.IL-1β= Interleukin-1β; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

Table 1. Influence of *Acacia nilotica* and/or γ-rays on right testis weight, sperm count and sperm motility of different rat groups.

Parameters	Rat groups			
	Control	*Acacia nilotica*	γ-irradiated	*Acacia nilotica*+ γ-rays
Right testis weight(g)	1.27 ± 0.05	1.29 ± 0.09	0.61 ± 0.04*,§	1.11 ± 0.03$^{*,§,#}$
Sperm count (million)	97.14 ± 21.22	99.2 ± 16.23	61.2 ±16.16*,§	78.57 ± 9.19$^{*,§,#}$
Sperm motility (%)	67.22 ± 4.23	68.12 ± 3.44	31.29 ± 2.58*,§	56.10 ± 3.32$^{*,§,#}$

Sperm count= Million/ ml of right caudal epididymis; *P< 0.05 as compared to control group; $^{§}P$<0.05 as compared to *Acacia nilotica* group; $^{#}P$< 0.05 as compared to γ-irradiated group.

Table 2. Influence of *Acacia nilotica* and/or γ rays on chromosome aberration frequencies in rat bone marrow cells of different rat groups.

Parameters	Rat groups			
	Control	*Acacia nilotica*	γ-irradiated	*Acacia nilotica*+ γ-rays
Abnormal metaphase Chromosomal aberrations	1.00±0.00	1.17±0.31	21.17±0.87*,§	11.50±1.36$^{*,§,#}$
Ring	0	0	3.17±0.48*,§	1.83±0.31$^{*,§,#}$
Dicentric	0	0	5.17±0.48*,§	2.33±0.42$^{*,§,#}$
Chromosome break	0.33±0.21	0.50±0.22	8.00±0.73*,§	4.33±0.84$^{*,§,#}$
Chromatid break	0.67±0.33	0.83±0.31	9.00±0.58*,§	5.83±0.79$^{*,§,#}$

*P< 0.05 as compared to control group; $^{§}P$< 0.05 as compared to *Acacia nilotica* group; $^{#}P$< 0.05 as compared to γ-irradiated group.

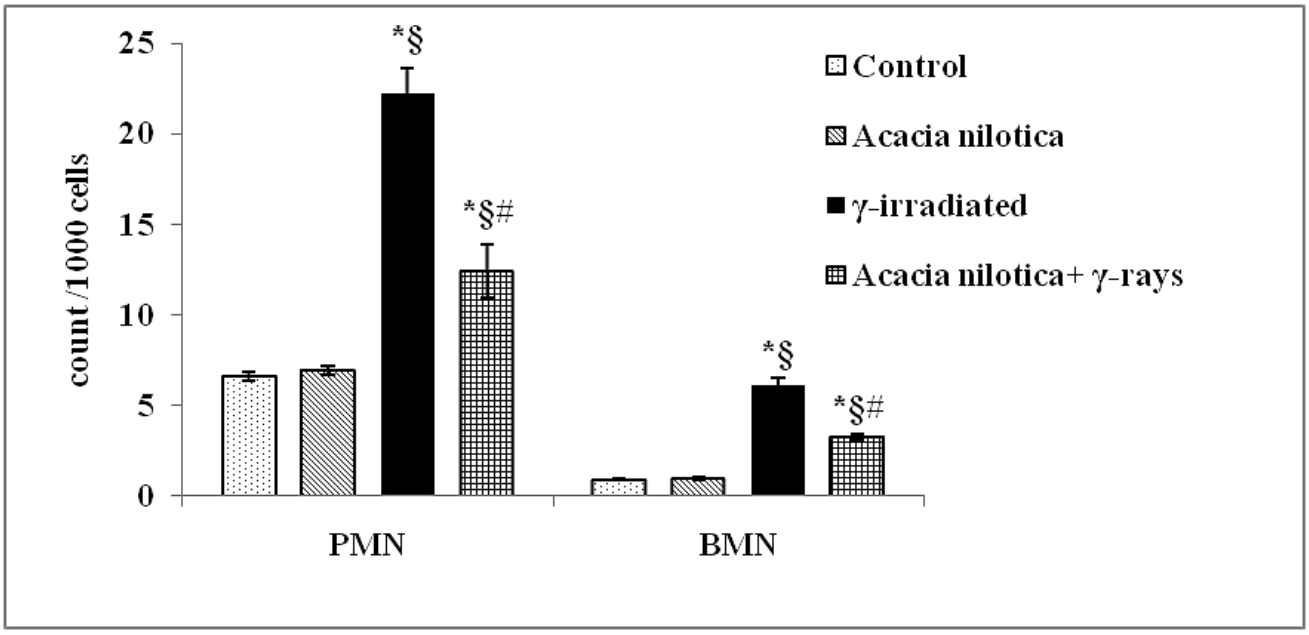

Figure 9. Influence of Acacia nilotica and/or γ-rays on PMN in rat peripheral-blood and BMN in bone marrow cells of different rat groups.RBC's= Erythrocyte cells; PMN= Peripheral blood micronucleus; BMN= Bone marrow micronucleus; *P< 0.05 as compared to control group; §P< 0.05 as compared to Acacia nilotica group; #P< 0.05 as compared to γ-irradiated group.

4. Discussion

Radiation uses are exponentially growing in modern society since industry development and machine technology worldwide. The impact of radiations on community health is well documented but little research has been done on radiation pollutants and male reproductive organs causing temporary infertility (Ahmed and Tawfik 2010). Gamma radiation can prompt cytogenetic disorders (Balajee and Hadjidekova 2021) and alter the oxidant/antioxidants particles and inflammatory cytokines markers in blood and tissues.

Acacia species significantly improved damaging influence on testes of rats (Al-Doaiss and Al-Shehri 2020), by reducing the inflammation and the oxidative expressions.

The rise of MDA could result in free radicals' productions in rat cells following γ-rays-exposure by injured the lipids membranes that are profuse in the testicle tissues (Soliman et al. 2020) besides, the MDA as an end-products of lipid peroxidation, directly threaten the activity of the testicle tissues (Tanriverdi et al. 2020). Furthermore, the lessening in TAS could be ascribed to the boosted use of the antioxidant system as an endeavor to trap the free radicals generated after γ-rays-exposure (Ibrahim et al. 2019). Besides, the exhaustion of TAS might be related to the exhaustion of the antioxidant enzymes; GSH, SOD, CAT and GPx documented in this study (Abdel-Magied and Shedid 2020).

Further, the authors found that *Acacia nilotica* had protecting and therapeutic effects on γ-rays-induced testes damage and suggested that it moderates the level of the oxidants end product; MDA therefore prevents the testis oxidative pressure harm and enhances the activity of the antioxidant protection pattern.

It was demonstrated that *Acacia hydaspica* ethyl acetate extract; an Acacia species enhances the antioxidant protection mechanisms, restores the level of testosterone hormone, suppresses DNA damages, and confirms a protected role against free radical mediated testis disorders (Nasir et al. 2020). On contrary, the Acacia nilotica powder crude extract at a dose (200 mg/kg rat, daily) used for 8 weeks revealed a reduction in sperm count and motility as well as an elevation of the morphological abnormalities and these alterations were irreversibly in Wistar rats (Lampiao 2013). It is clear that the huge dose and the long treatment period are the reason for these results. On the other hand, 0.2% Acacia nilotica honey enhances the post-liquefy quality of frozen buffalo sperms and is proved as an alternative to antibiotics via its antimicrobial activity (Nasreen et al. 2020). In addition, Gum Arabic could be used as an alternative to egg

yolk in the protection and preservation of frozen stallion sperm, it reserves sperm motility, shape, vitality and fertility (Ali et al. 2018).

Numerous studies have indicated that γ-rays-exposure impacts testicle functions, integrity and spermatogenesis (Said et al. 2020). Gamma rays cause impairments in the normal function and uptake, which might lead to genetic toxicity, and injury of radiosensitive cells (Dowlath et al. 2021).

GSH has a vital role in sperm integrity and construction (Altamimi et al. 2021). Moreover, SOD and CAT keep sperms against lipid oxidation and oxygen tension (Khalil et al. 2021). SOD dismutases the oxygen toxicity ($-O_2$) to form hydrogen peroxide (H_2O_2), whereas CAT expels the H_2O_2. GPx is a vital enzyme in the cell membrane scavenging mechanism and protection of sperm, morphology, motility, integrity, and division (ErfaniMajdet al. 2021).Thus, in the present work, the significant rise of MDA and drops in the antioxidant enzyme activity, comprising GSH, SOD, CAT, and GPx propose a decline in the testosterone hormone and a disruption in the spermatogenesis post-γ-rays-exposure. Besides, the disruption of the antioxidant system resulting in falling of Leydig cells to produce testosterone (Chung et al. 2021). Furthermore, it was demonstrated that Leydig cells are the central target for the adverse influence of γ-rays-exposure on male reproduction organs (Abdel-Magied et al. 2019). The methanolic bark extract of Acacia species was useful in alleviating the benzo(a)pyrene-prompted oxidative tension and inflammation in the mouse lungs, it reduces the anti-oxidant activity of detoxifying enzymes; SOD, CAT and GPx, and GSH content (Afsar et al. 2020; El-Garawani et al. 2021).

Cytokines are a group of glycoproteins, that acts as an immunomodulatory defense reaction to testis tissue damage in the mammals via generating signaling particles among immune system leading to stimulation of additional inflammatory intermediators (Olugbodi et al. 2021). In the present article, the rise of the inflammatory cytokines (TNF-α and IL-1β) levels in the testicle tissues after γ-rays-exposure could be deduced by the correspondence between oxidative pressure and inflammation process, each one can be certainly initiated by the other (Abdel-Magied et al. 2019). Ionizing radiations were reported to decrease total sperm count, motility, and the testis-weight in rats (Qin et al. 2019). Oxidative pressure is a demarcated by numerous intermediators of rat infertility, which lead to sperm dysfunction in laboratory rodents and humans (Pintus et al. 2021).

Acacia hydaspica ethyl acetate extract protects against cisplatin-induced testis dysfunction leading to infertility and oxidative pressure in rats, it restores the enzymatic activities, testosterone level, MDA content, GSH, TNF-α, and DNA and spermatogenesis damages (Afsar et al. 2017).

The micronucleus (MN) assay is a principal assessment run to estimate the genotoxic perspective of an animal, it rises from acentric chromosome fragments during the sequence of nuclear division (Guo et al. 2020). These MN cells persist for a longer duration in the peripheral blood and in bone marrow of rats (Huo et al. 2020). In addition, the core action of the anti-mutagenic compound on DNA arises via the generation of free radicals, which in turn may initiate numerous kinds of genotoxic impairment. Amongst these, a rise in the level of MN is predictable (Turkez 2011).

BMN is a display of DNA-damage. It is a genetic toxicity endpoint and any decline in its rate gives a sign of the anti-genotoxicity of a specific composite (Valencia-Quintana et al. 2021). The rise of the MDA content in the γ-rays-exposed rats which performs adduct with cellular-DNA (Shabeeb et al. 2019), leads to DNA destruction and later rise the PMN that is obvious in the present study. A possible elucidation for the modulator influence of *Acacia nilotica* on the BMN and PMN created by γ-rays-exposure is that such composites catch the free radicals produced during the mutagen transformation (Abduljawad 2020).

5. Conclusions

The present article conducts to reveal the moderating influence and application of *Acacia nilotica* to protect the testicle ultra structure and functions in rats after γ-rays-exposure which great approach gained if successfully verified. The data revealed a rise of MDA level concomitant with a significantly reduced activity of TAS in the rat blood plasma. The rise in figures of CAs that detected in γ-rays-exposure group is noticeable and considerably decrease in *Acacia nilotica*+ γ-ray groups contributed to *Acacia nilotica* properties.

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