Pakistan J. Zool., pp 1-11, 2026. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230323150327

Protective or Therapeutic Efficacy of *Tribulus terrestris* on γ-Rays Induced Testicular Damage in Rats

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Article Information
Received 23 March 2023
Revised 25 March 2026
Accepted 08 April 2026
Available online 28 August 2026 (early access)

Authors' Contribution
AE conceived, designed the study, analyzed the data and performed pathological studies. ST, NR and WK performed biochemical studies and analyzed the data. All authors prepared the manuscript.

Key words
***Tribulus terrestris*, Testis disorders, γ-rays, Rat**

ABSTRACT

Tribulus terrestris (*T. terrestris*) is a sexual tonic. The probable protective or therapeutic effect of *T. terrestris* against γ-rays-induced testicular toxicity in rats was investigated. The study comprised 5groups; a control (2ml vehicle), *T. terrestris* (100mg *T. terrestris* group/kg), γ-rays group (vehicle+7Gy γ-rays), *T. terrestris*+γ-rays and γ-rays+*T. terrestris* group. All groups were treated for 28days. Oxidant-antioxidant markers, serum sexual hormones, testis and sperms characteristics and histopathological examinations were performed. It was found that γ-rays adversely affected the oxidant/antioxidant parameters; Malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant status (TAS), 17β-hydroxysteroid dehydrogenase (17β-HSD), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the testis tissue, testis and sperms characteristics, and serum hormone levels. Also, γ-rays group showed degenerative alterations in spermatocytes, serous, fibrinoid exudate and oedema in interstitial tissues with the dissociation of spermatogonial cell layer. *T. terrestris* administration pre- and post-γ-rays resulted in an increase in the serum hormones, GSH, TAS, 17β-HSD, SOD, CAT, and GPx levels, a decrease in MDA level, and improvement of the testis and sperms characteristics. To conclude, to an extent, the *T. terrestris* was protective and therapeutic against γ-rays-induced testicular-damage. It may be useful to the patients who submit to radiotherapy.

INTRODUCTION

Reducing the extended-term toxicity of testicular malignancies treatment is essential to sustain life's quality. This treatment includes radiotherapy which induces testicular damage that arises from the up production of reactive oxygen species (ROS) and a deterioration of the antioxidant system (Aydogdu *et al*., 2019; Dehdari-Ebrahimi *et al*., 2023). Radiotherapy elicits oxidative stress (Dil *et al*., 2023), that plays an important role in male reproductive dysfunction (Akram *et al*., 2023). Radiation-induced testicular damage is dose-dependent (Xiong *et al*., 2022). Eventually, γ-rays considerably exaggerate the testicle abnormalities in spermatogenesis, decrease primary spermatocyte and spermatid production, reduce sperm count, augment spermatozoa abnormalities, alter seminiferous tube and lumen diameters, thicken the Leydig cells, increase epithelium height and apoptosis of germ cells, and cause the loss of testis weight (Said *et al*., 2020).

Antioxidants deactivate ROS and preserve normal cell function (Hong and Qin, 2023). Thus, the use of radioprotector can inhibit radiation-induced testicular toxicity. *T. terrestris* is a novel natural healthy antioxidant, anti-inflammatory and antibacterial preparation for the research and development of pharmaceutical and food industries (Ariyan *et al*., 2021). Several substances with different biological activities and biochemical structures have been recognized in the dry extract of *T. terrestris* including steroidal saponins (protodioscin), phytosterols, flavonoid compounds, tannins, and terpenoids (Figueiredo *et al*., 2021). Nevertheless, data indicate that protodioscin

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0030-9923/2026/0001-0001 $ 9.00/0

has unique biological activity that could be attributed to the major biological actions related to *T. terrestris* in male and female reproductive systems (Abarikwu *et al*., 2020; Ghanbari *et al*., 2021).

To the best of our knowledge, there is no previous study concerning the radioprotective and therapeutic potentials of *T. terrestris* on testicular injury. In this work, we examined whether or not *T. terrestris* administration could improve the protective or therapeutic efficacy against oxidative stress and testicular toxicity induced by γ-rays in rats using biochemical, testis and sperm characteristics, morphometric and histopathological investigations.

MATERIALS AND METHODS

Animals and housing

30 rats (6 rats/ group), 6-month-old (250–280 g) were obtained from the Egyptian organization for biological product and vaccines, Helwan, Cairo, Egypt. The animals were kept in well ventilated plastic cages at temperature; 22 ± 2 °C, humidity; 50–60% and a 12 h light/dark cycle environment at the NCRRT, Nasr City, Cairo, Egypt. They were nourished using a commercial normal standard pellet diet and clean water *ad libitum*. Before the experiment, they were kept in the cages for 3 days. The trial protocol used on the animals was in agreement with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication no. 85723, revised 1996) and approved by the local ethical committee on animal research; the Research Ethics Committee (REC- NCRRT), protocol number; 26A, valid from 31/10/2020.

Irradiation technique

The γ-rays were delivered by a Canadian gamma cell-40 treatment unit at the NCRRT, Nasr City, Cairo, Egypt. Rats were treated with a single dose of whole-body 7 Gy at a dose rate of 0.38 Gy/ minute at the time of experimentation. This dose represented the sub-lethal dose for rats according to the study by Rosen *et al*. (2020). Furthermore, the dose was sufficient to induce testicular damage (Gawish *et al*., 2020). Moreover, Ibrahim *et al*. (2019) found that gamma radiation (7 Gy as a single dose) induced testicular toxicity in rats. In both articles, it was established that it was a repairable dose. Animals didn't receive anesthesia before irradiation.

Chemicals

The *Tribulus terrestris*-VEMOHERB® PT (Vemo-Ltd, Sofia, Bulgaria), dry extract produced from whole parts of the fruit that are harvested in the flowering period. It is an herbaceous perennial plant from the Zygophyllaceae family. The supplier noted that the standardized dry extract contains furostanol saponins up to 60%; (30-45% protodioscin), steroid saponins (furostanol and spirostanol), and sapogenins. Also, it contains flavonol glycosides like astragalin, tribuloside, flavonoids (5-10% rutin), tannic compounds (5-10% tannin) and oils (Figueiredo *et al*., 2021). The extract was stored in a dry and cool room, away from direct light.

It was used in a single dosage of 100 mg/ kg/ day for the following 28 days. The selected dose of *T. terrestris* was chosen based on a previous protocol described by Surender *et al*. (2012), who tested both 50 and 100 mg of an extract of the dried fruits of *T. terrestris*/kg rats/day for 28 days as a sexual enhancer in the management of sexual dysfunction in males. They found that the dose of 100 mg resulted in reversing sexually sluggish male experimental rats. So, the author concluded that there was no need to evaluate other doses of *T. terrestris*. All other chemicals used were of analytical purity grade and they were obtained from Sigma-Aldrich Co., USA. The drug treatment was dissolved in volumes of 2 ml distilled water as a vehicle and was administrated orally.

Grouping and T. terrestris *administration*

The rats included in the study were divided into 4groups: Control group: The rats received the vehicle (2 ml distilled water), *T. terrestris*: The rats received *T. terrestris* (100 mg/kg), γ-rays: The rats received the vehicle, then after ½hour of the last dose exposed to whole-body 7Gy γ-rays, *T. terrestris* + γ-rays: The rats received *T. terrestris* dosages then, after ½hour of the last dose, exposed to 7Gy γ-rays and γ-rays+*T. terrestris*: The rats exposed to 7Gy γ-rays then, after ½ h they received *T. terrestris* dosages.

Experimental procedures and treatments

After the acclimatization and treatment periods (28 days), all animals were sacrificed by cervical dislocation on day 31. Blood samples were collected under standard laboratory conditions. Cauda epididymis and testes organs were dissected washed with ice-cold saline and then testes were weighed. Immediately the testicular tissue samples were collected and distributed into 2 quotas for biological and histopathological studies.

Testicular tissue

The testicular tissues were cut into small parts using scissors and homogenized in (1;10, w/v) of Triose HCI buffer; 50 mM, pH 7.4 by Homogenizer (MNW−302, Poland) for 2 min. The homogenates were then centrifuged at 3000xg for 8 min. The testis samples were stored at −80 °C for oxidative-stress parameters.

Measurement of oxidative stress markers

In the testicular tissue samples, total antioxidant status (TAS) and 17β-hydroxysteroid dehydrogenase (17β-HSD) were estimated according to Erel (2004) and Jarabak *et al*. (1962). Levels of malondialdehyde (MDA) and reduced glutathione (GSH), activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes were measured using a commercially accessible kit (Zellbio GmbH, Germany) according to the manufacturer's procedures. The absorbance was read at 420 m, 412 nm, 535 nm, 405 nm, and 420 nm, respectively.

Sperm analysis

Right cauda epididymis was set in a Petri-dish with 10 ml of 0.1 M phosphate-buffered saline (PBS; pH 7.4) for sperm count and sperm motility evaluation. Sperm analysts were performed using the methods previously reported in the study by Sahin *et al*. (2016). Sperm count (1000 sperm/ rat) was done by the hemocytometer under an ordinary microscope. A cover slide was placed on the hemocytometer then, a drop of 10µl from caudal epididymis sperm suspension loaded under the cover slide. Also, hemocytometer was used for sperm motility (1000 sperm/ rat) study; a cover slide was placed on the hemocytometer then, a drop with 10µl of caudal epididymis sperm suspension was loaded under the cover slide. Sperm viability was evaluated by the eosin-nigrosin technique using a light microscope, where unstained sperms were calculated as viable and stained sperms were calculated as non-viable. The viability of sperm was pronounced in percentage means. To evaluate the morphological aspect of the sperms, the epididymis sperm suspension (50 µl) was loaded on a glass slide, fixed with 70% methanol and then stained with toluidine blue stain. By using a usual optical microscope (Olympus, Tokyo, Japan), abnormal sperms were calculated, and the sperms without tails, with twisted tails or coiled, or double or atypical heads were counted as abnormal sperms (Khoshbakht *et al*., 2021).

Serum hormones level

Serum testosterone level was measured by using coated-tube radioimmunoassay rat testosterone kit (No. TKTT1, Los Angeles, USA). Luteinizing (LH) and follicle-stimulating hormones (FSH) levels were evaluated using ELISA hormones assay commercial kits from BioVendor, Czech Republic. Rat gonadotropin-releasing hormone (GnRH), using MyBiosource kit, Catalog No.; MBS762089, San Diego, USA.

Histopathological examination

The left testis was cleaned from the epididymis and fixed in a 10% neutral formalin solution for 72 h. The testis tissue was dehydrated by graded alcohol solutions (70–100%). The tissue was cleaned in xylene and get fixed in paraffin blocks. Then, the tissue slides were cut into 4–micron cross-sections, prepared from the upper, mid, and lower parts of the testis. The tissue slides were stained with hematoxylin and eosin (H&E) stain, according to Suvarna *et al*. (2013) for histopathological analyses and examined under a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

Data was represented as mean ± standard error of the mean (S.E.M). One-way analysis of variance (ANOVA) was employed and followed by a post hoc Fisher's PLSD-test. The level of statistical significance was set at $p< 0.05$ (Snedecor and Cochran, 1989).

RESULTS

T. terrestris did not produce any behaviour alterations or death in the experimental animals during the whole observation period. As shown in Table I, there was no significant difference between the control group and the *T. terrestris* group (dried *terrestris* fruits extract alone) as regards all assessed markers in testis tissue of the rats as shown in Table I.

Table I. Effect of *T. terrestris* on levels of oxidant/ antioxidants parameters levels of X-rays induced testicular damage in rats.

Groups	Control	*T. terrestris*	γ-rays	*T. terrestris* + γ-rays	γ-rays + *T. terrestris*
MDA (nmol/mg protein)	15.45 ± 0.71^{aa}	15.21 ± 0.69^{aa}	33.14 ± 1.92^{cc}	14.63 ± 0.54^{ad}	19.58 ± 0.73^{de}
GSH (µmol/g protein)	21.26 ± 1.06^{aa}	21.77 ± 1.07^{aa}	10.34 ± 0.63^{cc}	18.15 ± 0.81^{dd}	17.46 ± 0.77^{dd}
TAS (mmol H_2O_2 Eq/g protein)	0.65 ± 0.03^{aa}	0.67 ± 0.03^{aa}	0.43 ± 0.03^{cc}	0.61 ± 0.02^{dd}	0.60 ± 0.02^{dd}
SOD (U/mg protein)	89.58 ± 4.35^{aa}	88.51 ± 4.46^{aa}	38.42 ± 2.04^{cc}	81.25 ± 3.66^{dd}	80.14 ± 3.26^{dd}
CAT (U/mg protein)	0.71 ± 0.04^{aa}	0.69 ± 0.04^{aa}	0.28 ± 0.02^{cc}	0.66 ± 0.03^{dd}	0.65 ± 0.03^{dd}
GPx (U/mg protein)	21.12 ± 0.86^{aa}	20.03 ± 0.85^{aa}	10.23 ± 0.51^{cc}	18.12 ± 0.72^{ad}	17.74 ± 0.61^{de}

Control group (2ml vehicle), *T. terrestris* group (100mg *T. terrestris*/kg), γ-rays group (vehicle+5Gy γ-rays), *T. terrestris*+γ-rays group (100mg *T. terrestris*+5Gy γ-rays) and γ-rays+*T. terrestris* group (5Gy γ-rays+100mg *T. terrestris*). Values are expressed as mean±S.E.M. (n= 6). Different superscript letters (a,b,c,d,e) in the same row indicate significant differences at $p< 0.05$. MDA, malondialdehyde; GSH, glutathione; TAS, total antioxidant status; SOD, superoxide dismutase; CAT, catalase and GPx, glutathione peroxidase.

Table II. Effect of *T. terrestris* on weight of testis, and catachrestic of sperms in X-rays treated rats.

Groups	Control	*T. terrestris*	γ-rays	*T. terrestris* + γ-rays	γ-rays + *T. terrestris*
Right testis weight (g)	1.08 ± 0.053^{aa}	1.09 ± 0.061^{aa}	0.54 ± 0.026^{cc}	1.08 ± 0.034^{aa}	1.07 ± 0.044^{aa}
Sperm count (million)	108.30 ± 5.182^{aa}	146.20 ± 5.841^{bb}	68.20 ± 4.093^{cc}	89.10 ± 3.293^{dd}	87.60 ± 3.134^{dd}
Sperm motility (%)	71.10 ± 3.493^{aa}	79.30 ± 3.192^{aa}	39.30 ± 2.181^{cc}	63.10 ± 2.162^{dd}	61.10 ± 2.224^{dd}
Sperm abnormality (%)	1.22 ± 0.042^{aa}	1.12 ± 0.028^{aa}	31.56 ± 1.362^{cc}	28.81 ± 1.122^{cc}	29.86 ± 1.134^{cc}
Sperm viability (%)	84.92 ± 3.041^{aa}	85.82 ± 3.172^{aa}	51.44 ± 2.391^{cc}	74.22 ± 2.641^{dd}	76.58 ± 2.712^{dd}

See Table I of statistical details and details of experimental treatments.

In rat testis tissue, MDA level was significantly elevated, while GSH, TAS, SOD, CAT, and GPx levels were significantly declined in γ-rays group when compared to the control group and *T. terrestris* group (Table I). On the other hand, pre usage of *T. terrestris* in *T. terrestris* + γ-rays group stimulated a significant reduction in MDA level when compared to the γ-rays group and there was no significant difference observed when compared to the control group and *T. terrestris* group. In the γ-rays + *T. terrestris* group, a significant decrease in MDA level was observed when compared to the γ-rays group. There was still a significant difference observed in MDA level when comparing the former group to the control and *T. terrestris* group.

There was a significant augmentation in GSH, CAT, and GPx levels in both *T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group when compared to γ-rays group. Also, there was a significant difference noted as regards the mentioned parameters when compared to *T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group, Table II. The GPx activity was significantly increased in both *T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group when compared to the γ-rays group (Table I). The GPx activity in the *T. terrestris* + γ-rays group showed no significant difference when compared to the control group and *T. terrestris* group but γ-rays + *T. terrestris* group showed a significant difference when compared to the control group and *T. terrestris* group (Table I). However, none of the treatment groups (*T. terrestris* + γ-rays and γ-rays + *T. terrestris*) showed any significant change compared to each other, Table I.

There was no significant difference between control group and *T. terrestris* group as regards right testis weight character. In the γ-rays group, the animals exhibited a significant decrease in their right testes weight when compared to the control group and *T. terrestris* group (Table II). Twenty-eight days pre or post γ-irradiation (*T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group), the animals exhibited a significant increase in their right testes weight when compared to the γ-rays group until they reached control weight in the control group and there was no significant difference when compared to the control group and *T. terrestris* group (Table II).

Rats that were supplemented with *T. terrestris* group revealed significant higher sperm count and sperm motility percentage when compared to the control group. However, the γ-rays group showed significant decreases in sperm count and sperm motility percentage when compared to the control group and *T. terrestris* group. Both pre- and post-treatment with *T. terrestris* (*T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group) significantly increased the sperm count and sperm motility percentage when compared to γ-rays group, but there was a significant difference when compared to the control group and *T. terrestris* group, Table II. A dramatic increase in the percentage of sperm abnormality was detected in the γ-rays group, thereafter the significant increase was constant in the *T. terrestris* + γ-rays group and the γ-rays + *T. terrestris* group and there was a significant difference as regards these parameters when compared to the control group and *T. terrestris* group (Table II). Sperm viability percentage in γ-rays group showed a significant decrease when compared to the control group and *T. terrestris* group then, its percentage increased significantly in both the *T. terrestris* + γ-rays group and the γ-rays + *T. terrestris* group but still showed significant difference when compared to control group and *T. terrestris* group (Table II). However, none of the treatment groups (*T. terrestris* + γ-rays and γ-rays + *T. terrestris*) showed a significant change compared to each other (Table II).

In the *T. terrestris* group, administration of *T. terrestris* caused a significant increase in testosterone level but, the γ-rays group revealed a significant decrease in testosterone level when compared to the control group and *T. terrestris* group. The testosterone level in both the *T. terrestris* + γ-rays group and the γ-rays + *T. terrestris* group showed significant increases when compared to γ-rays group till reached to the control range but still showed significant decreases when compared to the *T. terrestris* group (Table III).

The 17β-HSD activity was significantly declined in γ-rays group when compared to control group and

Table III. Effect of *T. terrestris* on different hormonal levels and 17β-HSD activity in tests of rats expressed of X-rays.

Groups	Control	*T. terrestris*	γ-rays	*T. terrestris* + γ-rays	γ-rays + *T. terrestris*
Testosterone (ng/ml)	1.86 ± 0.062^{aa}	3.41 ± 0.131^{bb}	0.47 ± 0.019^{cc}	1.68 ± 0.053^{aa}	1.71 ± 0.061^{aa}
LH (ng/ml)	1.04 ± 0.032^{aa}	1.26 ± 0.051^{aa}	0.37± 0.011^{cc}	0.66 ± 0.024^{dd}	0.64 ± 0.016^{dd}
FSH (ng/ml)	12.11 ± 0.523^{aa}	11.91 ± 0.512^{aa}	6.81 ± 0.281^{cc}	10.54 ± 0.412^{dd}	9.82 ± 0.392^{dd}
GnRH (ng/dl)	6.82 ± 0.261^{aa}	6.64 ± 0.272^{aa}	4.24 ± 0.213^{cc}	5.28 ± 0.221^{dd}	5.11 ± 0.212^{dd}
17β-HSD (nmol/mg protein)	15.52 ± 0.622^{aa}	15.81 ± 0.601^{aa}	7.41 ± 0.362^{cc}	13.08 ± 0.522^{aa}	13.54 ± 0.511^{aa}

For other details see Table I. LH, Luteinizing hormone; FSH, follicle-stimulating hormones; GnRH, gonadotropin-releasing hormone; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

T. terrestris group (Table III). Then the 17β-HSD activity was significantly increased in both the *T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group when compared to the γ-rays group (Table III) but, there was no significant difference in 17β-HSD activity observed when compared to the control group and *T. terrestris* group. However, none of the treatment groups (*T. terrestris* + γ-rays and γ-rays + *T. terrestris*) showed significant changes when compared to each other, Table III.

The LH, FSH, and GnRH assays revealed significant decreases in the γ-rays group when compared to the control group and *T. terrestris* group. The three hormones showed significant augmentations in both the *T. terrestris* + γ-rays group and the γ-rays + *T. terrestris* group and also showed significant differences when compared to the control group and *T. terrestris* group (Table III). However, none of the treatment groups (*T. terrestris* + γ-rays and γ-rays + *T. terrestris*) showed significant change as regards these hormones when compared to each other (Table III).

Histopathological finding

In control the group and *T. terrestris* group, testes were walled from the exterior by a thick connective tissue capsule called tunica albuginea, inside it several circular or ovoid structures with thick walled structures called seminiferous tubules which were lined with a specialized stratified epithelium called the germinal epithelium which comprised the spermatogenic and supporting or sertoli cells. These cells rested on a thin basement membrane. Between the tubules in the interstitial tissue, consisting of fibroblasts, blood vessels, nerves, lymphatic and interstitial (leyding) cells were observed (Fig. 1A, B). Testes in the γ-rays group, showed mild effects denoted by degenerative alterations in primary and secondary spermatocytes, serous fibrinoid exudate and oedema in interstitial tissues with dissociation of spermatogonial cell layer and spermatocytes in tubules Moreover, spermatogenic and sertoli cells of seminiferous tubules with little or complete absence of spermatozoa in addition to interstitial fibrosis, congested blood vessels were noticed in more severe cases of necrosis (Fig. 1C, D, E, F). While in the *T. terrestris* + γ-rays group, normal seminiferous tubules with adequate spermatozoa in the lumen without pathological deteriorating changes were observed. Some cases reveal normal testis with slight thickness of the interstitial tissues and little spermatozoa in lumen were noticed (Fig. 1G, H).

DISCUSSION

The oxidant-antioxidant stability is delivered by different types of oxidants that arise in the animals, and appropriate antioxidant mediators compensate for these oxidants (Salem *et al*., 2021).

The data demonstrated a significant damage in the rat testis, an up rise in oxidative stress and a reduction in antioxidants. In addition, the study was carried out to explore the link between γ-rays and oxidative stress related to testis dysfunction and structural changes in testicle tissue. Pre and post *T. terrestris* administration led to an enhanced cellular antioxidant system, decreased oxidative stress, improved testis function and ultrastructural integrity.

It has been established that mammalian testes are very sensitive to oxidative stress (Men *et al*., 2023). In the present study, it was shown that ionizing-radiation exposure of rats resulted in a significant rise in testicle MDA as well as a decline of the oxidant/antioxidant parameters; GSH, TAS, 17β-HSD, SOD, CAT, and GPx) levels. These results are similar to those of the other studies in rats and mice (Abdel-Magied *et al*., 2019; Aydogdu *et al*., 2019; Gawish *et al*., 2020; Gvilava *et al*., 2018; Ibrahim *et al*., 2019). In the pre-treatment group (*T. terrestris* + γ-rays), *T. terrestris* protected against γ-irradiation altered GPx activity till there was no significant difference observed when compared to control group and *T. terrestris* group. Further, the authors presented that *T. terrestris* had protective and therapeutic effects on γ-rays-induced testes injury and established that *T. terrestris* reduces the level of the oxidants product (MDA) thus preventing the testis oxidative stress injury and augmenting the activity of the antioxidant defence system. In the pre-treatment group (*T. terrestris* + γ-rays),

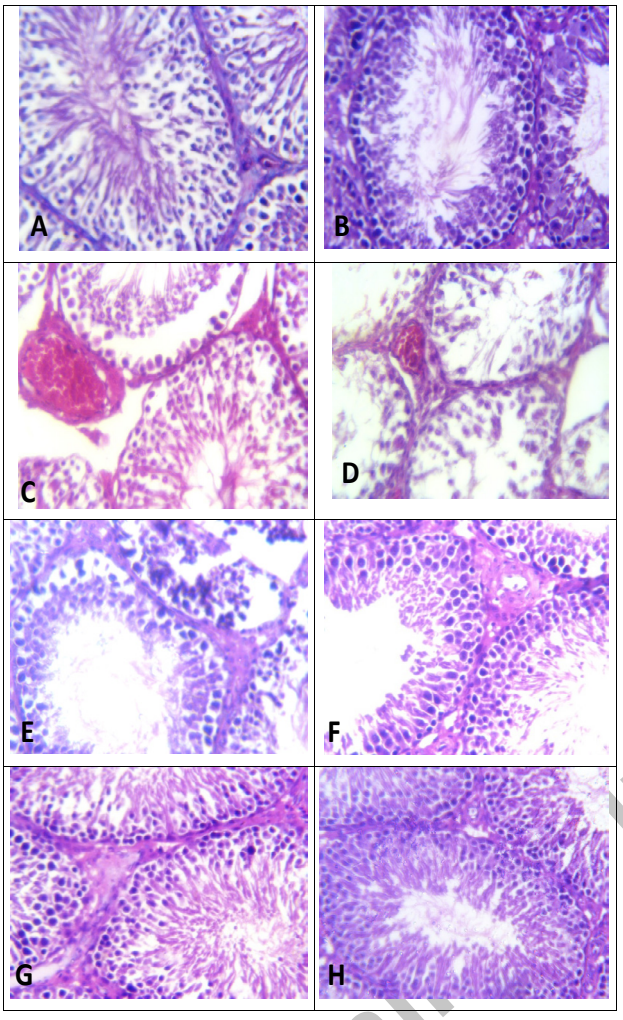

Fig. 1. Histopathological pictures of testes among experimental groups (H & E x400).
A, Testis of control group showing normal structure. B, Testis of *T. terrestris* group showing normal structure. C, Testis of γ-rays group showing edema, congested blood vessels in interstitial septa dissociation of spermatogonial cell layer and spermatocytes of tubules with little spermatozoa. D, Testis of γ-rays group showing degenerative changes and necrosis of spermatogonial cell layer and spermatocytes of tubules with tiny spermatozoa in lumen with thickness of interstitial tissues. E, degenerative changes and necrosis of spermatogonial cell layer and spermatocytes of tubules, with tiny spermatozoa in lumen. F, Testis of γ-rays group showing interstitial fibrosis, edema with absence of spermatozoa in lumen. G, Testis of γ-rays + *T. terrestris* group showing slightly thickness of interstitial tissues with normal seminiferous tubules and adequate spermatozoa in lumen. H, Testis of *T. terrestris* + γ-rays group showing normal seminiferous tubule with little spermatozoa in lumen.

T. terrestris protected against γ-irradiation altered MDA content till there was no significant difference when comparing to the control group and *T. terrestris* group. *T. terrestris* has free radical scavenging and antioxidant activities. It significantly increases all testis reproductive parameters and decreases MDA level in rats treated with malathion that induce free radical damage and male reproduction disorders (Salahshoor *et al*., 2020). Also, Ariyan *et al*. (2021) concluded that adding *T. terrestris* ethanol extract to goat epididymal sperms, significantly increased the percentage of total sperm motility and viability and significantly decreased the level of MDA concentration.

Determining TAS is a beneficial, fast and certainly routine technique to estimate the complex oxidative exploration of a disease (Bizoń *et al*., 2023). In the present study, the TAS indicator data revealed that *T. terrestris* protects/treats testis from γ-rays-induced oxidative stress (Shabeeb *et al*., 2019), moreover, *T. terrestris* is a novel natural antioxidant extract (Khalid *et al*., 2022), that protects against oxidation processes and exhaustion of antioxidant enzymes.

In the present study, we found that acute exposure to γ-rays group caused a decrease in 17β-HSD activity that may be related to the decline in serum testosterone level. The 17β-HSD enzyme plays an important role in the biological activity of steroid hormones such as testosterone (Rizwan *et al*., 2023). A decline in its activity causes a reduction in the serum testosterone level, total sperms count per epididymis, sperms viability and sperm motility, and a significant sexual dysfunction (El-Mazoudy *et al*., 2020; Soliman *et al*., 2019). However, in pre- and post-treatment groups (*T. terrestris* + γ-rays and γ-rays + *T. terrestris*), *T. terrestris* protected against γ-irradiation alter 17β-HSD activity till there was no significant difference when compared to control group and *T. terrestris* group. It has been established that Myricetin effectively lessened the Nonylphenol-induced oxidative stress and testicular injuries and reduced the 17β-HSD activity and testosterone level in male Sprague-Dawley rats (Ijaz *et al*., 2021). Also, Hygrophila auriculata Seeds reduced the Cyproterone Acetate induced testicular dysfunction. It reduced SOD, CAT, and testis-somatic markers in rats (Ghosh *et al*., 2023). In addition, the protodioscin present in *T. terrestris* extract increased serum testosterone levels and 17β-HSD activity, which might increase sperm production (Wang *et al*., 2019). These alterations could be involved with the histopathological findings detected in the testis.

In *T. terrestris* group, sperm count and percentage of sperm motility were significantly increased when compared to the control group. Recently, it was reported that *T. terrestris* improved the sperm quality of male rats

and goats (Aldaddou *et al*., 2022; Ariyan *et al*., 2021). Gamma radiations have been reported to decrease sperm motility and vitality, and the percentage of morphologically normal sperms in men (Wdowiak *et al*., 2020). Similarly, the whole body 5.5Gy gamma-radiation exposure cause a significant reduction in testis weight and sperm count in mice (Wang *et al*., 2021). Oxidative stress is defined as one of several mediators of male infertility, which causes sperm dysfunction in rats and mice (Dehdari *et al*., 2023).

Also, the sperm count, and percentage of sperm motility were increased significantly in rats treated with *T. terrestris* (Haghmorad *et al*., 2019). Moreover, in the γ-rays group, the animals showed a significant decrease in the right testes weight and a dramatic increase in the percentage of sperm abnormality when compared to the control group and *T. terrestris* group. *T. terrestris* had potential effectiveness in preventing damage in testis and epididymis sperm characteristics. The rats treated with *T. terrestris* (*T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group) revealed a significant increase in right testis weight, sperm count, and percentage of sperm motility and viability and a significant reduction of the percentage of sperm abnormality when compared to γ-rays group but still a significant difference was observed when compared to the control group and *T. terrestris* group except for testis weight that reached to the normal range. In agreement with the present data, it was shown that *T. terrestris* administration significantly improved testes weights, sperm count and percentage of sperm motility, abnormality, and viability (Haghmorad *et al*., 2019; Noh *et al*., 2020).

Gamma-rays' exposure significantly declined the sexual hormones; testosterone, LH, FSH, and GnRH in rat serum of γ-rays group. In harmony with the present data, it was reported that 5.5 Gy gamma rays reduced testosterone, LH, and FSH significantly in mice (Wang *et al*., 2021), and 8.7 Gy gamma rays reduced GnRH in zebrafish offspring (Hurem *et al*., 2018). Serum testosterone enhanced the testicular antioxidant enzymes activity and attenuated the degenerative alterations induced in the testes of intoxicated rats (Noh *et al*., 2020). The usage of *T. terrestris* augmented testosterone, LH, and FSH in diabetic rats with erectile dysfunction rat (Ștefănescu *et al*., 2021).

The extract of *T. terrestris* has been considered as a prospective stimulator of testosterone production (Morgado *et al*., 2023; Salahshoor *et al*., 2020). Moreover, *T. terrestris* supplement improves sexual function in male rats and the sexual performance in men (Aldaddou *et al*., 2022; Ebrahimpour *et al*., 2020).

In the present study, the histopathological findings of testicular tissue in all groups were in agreement with those recorded by biochemical, hormonal and testis and sperms functional analyses, Figure 1. The present histopathological findings were average and similar when the control group and the *T. terrestris* group were compared, where the testis showed the uniformity of the seminiferous tubules and the smoothness of the interstitial tissue. Said *et al*. (2020) stated that the cross-sections of the testis of normal control rats showed the consistency of the seminiferous tubules and interstitial tissue.

In contrast, the current study detected severe histopathological alterations in the testis of the γ-rays group. A deterioration in the base membrane of the seminiferous tubules of the testis, reduction in the total number of the spermatozoids, development of the seminiferous tubule vacuoles and degenerative-necrotic abnormalities in the epithelial cells. There was tubular atrophy, hyalinization and degeneration of the seminiferous epithelium of testis of γ-irradiated rats where γ-rays injured the seminiferous tubules and destructed the structures of the Leydig cells. The diagnostic indicators of radiation-induced testicular damage were reductions in epithelial height, seminiferous tubules' diameter and testis' weight and size and these findings were in line with previous similar studies (Akdere *et al*., 2015; Kamischke *et al*., 2003; Said *et al*., 2020). In addition, the increase in free radicals following γ-rays-induced testicular injury led to oxidative stress and deterioration of the antioxidant defence system as in related previous study (El-Mesallamy *et al*., 2018). However, these alterations were modulated and/ or significantly enhanced in *T. terrestris* + γ-rays group and γ-rays + *T. terrestris* group. In an earlier study, *T. terrestris* exerted a protecting influence against testicular injury induced by cadmium in rats where *T. terrestris* intermediated by its antioxidant activity and stimulating testosterone production (Rajendar *et al*., 2011). Adding, *T. terrestris* and Anacyclus Pyrethrum mixture enhanced the fertility indices in male Wistar rats and it alone alleviated Cypermethrin-induced reproductive injury in male Wistar rats (Haghmorad *et al*., 2019; Sharma *et al*., 2013). Moreover, *T. terrestris* improved the changes induced Cyclophosphamide toxicity in mouse testis, contribute to the presence of protodioscin (Pavin *et al*., 2018). Also, it enhanced ameliorated lead-induced inferiority in male rats (Aldaddou *et al*., 2022).

CONCLUSION

T. Tribulus was able to exert a potential protective and therapeutic role against γ-rays induced testicle toxicity in rats. However, further studies are required for other animals and humans regarding the clinical trials to confirm the potential medical use of *T. terrestris* and fully recognize its mode of action.

DECLARATIONS

Acknowledgements

We express our gratitude to our colleagues, the Health Radiation Research Department for providing laboratory conveniences, and members of the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority for providing the necessary irradiation facilities. This work was done in the NCRRT.

Funding

The study received no external funding.

Ethical approval

All experiments were performed in accordance with the Research Ethics Committee (REC, NCRRT) Number: 26A, valid from 31/10/2020.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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