Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 6 Year 2024 Page 258 -275

Testicular Dysfunction Via Uvb-Induced Hyperthyroidism And Prophylactic Glycyrrhizin Dose In Swiss Albino Mice

Shashank Shakyawal[1], Gayatri Rai[2] ,Zaved Ahmad[3], Payal Mahobiya[4*]

[1,2,4*]*Endocrinology Lab, Department of Zoology, Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh, 470003, India*
[3]*Cancer Biology Laboratory, Department of Zoology, Dr. Harisingh Gour Vishwavidyalaya, Sagar, (Madhya Pradesh), India*
000-0001-5723-5490; Email- shakyawal.shashank@gmail.com
0000-0002-8626-6779; Email- phd008gayatri@gmail.com
0000-0001-6612-3239; Email- zavedjmi@gmail.com
0000-0003-3454-7437; Email- 1607payal@gmail.com

***Corresponding author:** Payal Mahobiya*
**Email- 1607payal@gmail.com*

Abstract

This study explores the protective potential of glycyrrhizin, a compound from licorice root, against UVB-induced testicular dysfunction. UVB exposure disrupts hormonal balance, potentially leading to impaired male fertility. Glycyrrhizin's established antioxidant and anti-inflammatory properties suggest its efficacy in mitigating these detrimental effects.
A rodent model will be employed, with controlled UVB irradiation mimicking hyperthyroidism-induced testicular damage. Testicular function will be assessed via sperm quality and motility. Additionally, oxidative stress markers (malondialdehyde) and antioxidant enzyme activity (SOD, CAT, GSH) will be evaluated. Histological examination will reveal potential alterations in spermatogenesis and seminiferous tubule structure.
A crucial aspect involves administering glycyrrhizin to a designated UVB-exposed group. Comparing parameters between control, UVB-exposed, and glycyrrhizin-treated groups will elucidate its potential to reduce oxidative stress and preserve testicular health.
This research aims to shed light on glycyrrhizin's protective role against UVB-induced testicular toxicity. By evaluating its efficacy in mitigating oxidative damage and preserving testicular function, this study can contribute valuable insights for developing therapeutic strategies to safeguard male reproductive health following UVB exposure. The findings may hold particular relevance for individuals with high occupational UVB exposure or undergoing phototherapy treatments utilizing UVB radiation.

Keywords: Hyperthyroidism, Radiations, Testicular dysfunction, Glycyrrhizin

Introduction

Research on radiation-induced oxidative stress highlights the impact on biological systems of disrupting the

cellular capacity for oxidizing agents and antioxidants and affecting molecular signaling pathways (**Yakymenko *et al*., 2016**). Reactive oxygen species (ROS) are produced through mitochondrial membrane depolarization and water hydrolysis, damaging DNA chains and influencing radiation-induced physical effects. Whole-body exposure causes injuries, pathologies, inflammation, and immunosuppression, with chronic exposure leading to cancer, skin redness, and organ damage (**Dowlath *et al*., 2021**). Oxidation is crucial for energy production, with mitochondria being key sites. Thyroid hormone dysregulation affects these sites, potentially causing decreased thyroid function (**Mircescu, 2008**).

UVB radiation causes oxidative stress in the skin, primarily affecting keratinocytes, which produce hydrogen peroxide (**Kalyanaraman *et al*., 2012**). This radiation stimulates inflammatory reactions, leukocyte infiltration, and mutations in vascular endothelia, making it five times more effective than UVA radiation (**Yogianti *et al*., 2014; Cadet *et al*., 2015**). UVB exposure in female Wistar rats causes thyroid alterations, follicular degeneration, and increased hormone levels, impacting brain activity through POMC gene expression (**Rai and Mahobiya, 2022**).

A study revealed that 2.4 GHz RF radiation from Wi-Fi equipment affects testicular function and histology in rats. Exposure to 2.4 GHz RF radiation led to decreased motility, concentration, testis weight, and seminiferous tubule diameter. An electromagnetic wavelength of 900 MHz also decreased the sperm count and caused ultrastructural deformities (**Dasdag *et al*., 2015**). With decreased bcl-2 expression and increased BAX, cytochrome C, and active caspase-3 expression, sperm apoptosis was observed. Testosterone levels decreased in exposed rats, and local tubular injuries and Leydig cell vacuolization were observed. Caspase-3 enzyme activity was also increased in the radiated group (**Liu *et al*., 2015**).

The hypothalamus-pituitary-thyroid axis and the hypothalamus-pituitary-gonadal axis work together to influence male reproduction. Thyroid hormones, particularly T3, play a crucial role in sperm production, quality, and sexual function. Low thyroid function (hypothyroidism) is linked to reduced sperm count and motility, while high thyroid function (hyperthyroidism) can disrupt sperm development and structure (**Darbandi *et al*., 2018; Sahoo, 2012**). Furthermore, thyroid hormones affect testosterone production and proteins that bind sex hormones (**Ikegami *et al*., 2019**). Fortunately, treating thyroid problems can often reverse these negative effects on male fertility and sexual health.

The principal component of licorice root, glycyrrhizin, has a promising array of therapeutic properties. Studies have revealed its anti-inflammatory, antioxidant, and immunomodulatory effects (**Jayaprakasam *et al*., 2009; Li *et al*., 2011**). Glycyrrhizin may improve sperm quality, protect testes from toxins, and potentially mitigate neuroinflammation associated with neurodegenerative diseases (**Sakr and Shalaby, 2014**). These findings warrant further investigation into the potential of glycyrrhizin as a therapeutic agent.

In this study, we aimed to determine the potential of glycyrrhizin to mitigate testicular toxicity caused by UVB radiation-induced hyperthyroidism in mammals. Testicular function and male fertility are vulnerable to hyperthyroidism, and identifying protective agents is crucial. Glycyrrhizin, a natural flavonoid found in *Glycyrrhiza glabra*, has been shown to possess antioxidant and anti-inflammatory properties, making it a promising candidate for reducing hyperthyroidism-induced damage. By evaluating the modulatory effects of glycyrrhizin on mammals, this study could contribute to the development of therapeutic strategies to safeguard male reproductive health following UVB radiation-induced hyperthyroidism.

Materials and Methods

Animal Care-Handling and Ethics

The guidelines for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996), the EU Directive 2010/63/EU for animal experiments, the U.K. Animals (Scientific Procedures) Act, 1986 and related regulations, and the Dr. Harisingh Gour Vishwavidyalaya, Sagar's Department of Pharmaceutical Science were all followed when conducting any animal experiments. Sincere efforts were made to decrease the number of animals used and their level of suffering.

The research used 25±3 g of Swiss albino mice, aged 6–8 weeks, from an inbreeding colony. The rats were housed in climate-controlled environments with 12 hours each day of light and darkness.

The animals were fed standard mouse food and water (bought from Veterinary and Animal Husbandry College, Mhow). Additionally, as a preventative measure against infections, tetracycline water was given once every two weeks. Each group of six animals was housed in a plastic cage for the duration of the trial.

Chemicals
Analytical-grade chemicals and reagents were used. The supplier of glycyrrhizin was TCI (India) Pvt. The remaining chemicals were purchased from CDH (P) Ltd. in New Delhi, India, and included NBT, NADPH, methionine, reduced glutathione, and other compounds. Himedia in India was the source of the additional chemicals, which included hematoxylin, eosin, and thiobarbituric acid. The thyroid hormone receptor beta 1 (THRβ-1), caspase-3, cyclooxygenase 2 (COX-2), and poly (ADP-ribose) polymerase (PARP) antibodies were purchased from Emporium and Santa Cruz Biotechnology. A kit for inhibiting avidin and biotin was purchased from GeneTex under Cat. No. GTX30966.

UVB irradiation
The Swiss albino mice were exposed to UVB light, which has a wavelength of 280 nm and was produced in Germany. The irradiance was fixed at 2 hours every day for 15 days (**Akindele *et al*., 2014**)

Study Test Strategy
The animals were randomly separated into four groups with six mice in each group. A timeline of the dosage and experimental plan is shown in Fig. 1.

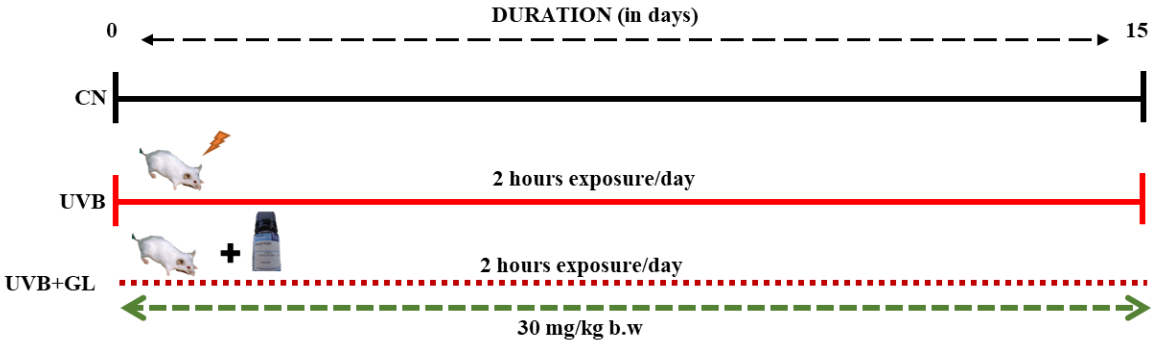


Fig. 1 Timeline showing the duration of the experiment and the radiation exposure time along with the concentration of GL

Group I: The animals received standard food and water ad libitum for 15 days (control group).
Group II: The animals were exposed to whole-body UVB radiation at 280 nm for 2 h every day for 15 days.
Group III: The animals were orally exposed to UVB radiation along with glycyrrhizin (at a concentration of 30 mg/kg body weight) for 15 days (**Li *et al*., 2011**).

Every animal in each of the three groups (I, II, and III) was watched closely for changes in weight, any indication of disease, offensive behavior, any obvious abnormalities, and death for a period of fifteen days. For the purpose of evaluating the biological, qualitative, and quantitative differences in testes, the animals were decapitated after being starved for the entire duration of the study. The arterial blood was then quickly extracted. The testes were removed via a transverse abdominal incision and weighed following the removal of the surrounding connective tissues.

Body weight and organ weight
The weights of the thyroid, testicles, and body were measured using an electronic balance (Sartorius, BP210 S). Body weight was measured before the experiment and every five days, while the weights of the thyroid and testicles were measured post experiment. The gonadosomatic index was calculated by utilizing both body weight and testicular weight **(Baghel & Srivastava, 2021).**

Sample collection and hormone measurement
Following the extraction of whole blood samples, the tubes were emptied and filled with an EDTA anticoagulant solution. After centrifuging the second tube for 15 minutes at 2200 rpm, the plasma was collected and stored at −20°C until the testis hormone levels were measured.

Follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and thyroid hormones were measured using ELISA kits (Calbiotech Inc., California, USA) (**Sachidhanandam *et al*., 2010**).

Samples for semen analysis

The entire spermatozoa count was determined by dissecting, chopping, and filtering the epididymis. A tiny amount was mixed with water and added to Neubauer's counting chamber. Each epididymis contains millions of spermatozoa (**Narayana *et al*., 2002**). After adding eosin to the suspension, it was dried. Under 1000X magnification, a thousand spermatozoa were examined for anomalies such as amorphous heads, banana heads, hammerheads, and pinheads (**Rai and Vijayalaxmi, 2001**).

Homogenate preparation for biochemical studies

Four mice from each group were slaughtered, and the thyroid and testicles were removed, washed with PBS solution, and stored at -20°C until use in the biochemical investigations. A homogenate (10% w/v) of the thyroid and testis extracts was made in 0.02 M Tris-Cl (pH 7.4). To extract the supernatant for the biochemical assays, the homogenized tissue was centrifuged for 30 minutes at 4°C at 12500 rpm.

Enzymatic and Nonenzymatic Activity Assessment

The remaining testis was used for analysis of lipid peroxidation (**Placer *et al*., 1996**), catalase (**Bergmeyer, 1983**), superoxide dismutase (**Beauchamp and Fridovich, 1971**), GSH (**Sedlak and Lindsay, 1968**), hydrogen peroxide (**Sinha, 1972**), glutathione reductase (**Carlberg and Mannervik, 1975**) and nitric oxide (**Griess *et al*., 1879**) levels in all the above groups.

Histopathological analysis

Whole-body perfusion through the heart was achieved using 0.02 M phosphate-buffered saline (PBS) and 4% PFA (**Stefanini *et al*., 1967**). The testes were extracted and then left in the fixative for two days. For hematoxylin and eosin staining, fixed tissues were treated, dried, and cut into 5-µm slices from paraffin-embedded testes. Images at 10× and 40× magnification were examined.

Immunofluorescence

Shakyawal *et al*.'s (**Shakyawal *et al*., 2023**) immunofluorescence method was used to detect THRβ-1, caspase-3, COX-2, and PARP immunoreactivity in the testes. Paraffin-embedded testicular tissue sections were deparaffinized and rehydrated. To remove the antigens (10 mM; pH 6.0), the sections were microwaved for 15 minutes while submerged in sodium citrate buffer. After the sections were brought to room temperature, they were rinsed with PBS. The sections were incubated with primary antibodies at 4°C overnight. The following antibodies were diluted 1:50 in PBS: THRβ-1 (Cat # 600-401-A96), caspase-3 (Cat # R37111), COX-2 (Cat # 376861), and PARP (Cat # sc-8007). The slides were incubated with the secondary antibody FITC at a dilution of 1:20 for 60 minutes at 4°C. Sections were counterstained with DAPI for four minutes at room temperature, and slides were then mounted using glycerin-based media. Immunofluorescence images were taken with an Invitrogen EVOS 5000 fluorescence microscope. Using ImageJ software, the immunoreactive cells were subjected to semiquantitative analysis for THRβ-1, caspase-3, COX-2, and PARP.

Statistical analysis

All the statistical analyses were conducted using one-way analysis of variance (ANOVA), and the results are shown as the means ± standard errors (SEs). Dunnett's test was utilized in GraphPad Prism (version 5.01) to compare the control group with the other groups. The significance thresholds were therefore established at *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

Results

Body Weight, Thyroid Weight and Thyroid Hormones

The body weight measured over the study period was analyzed using the mean body weight. There were significant differences in body weight between the II group and the I group (**$p<0.01$). The findings demonstrated that, in comparison to that in the I group, the thyroid weight in the II group decreased dramatically (**$p<0.01$) and that GL helped maintain the model in its normal state (Table 1).

The thyroid hormone levels of group II patients were notably greater than those of group I patients. TSH levels significantly decreased, while T3 and T4 levels significantly increased in the II group relative to those in the I group. Following UVB radiation exposure-induced hyperthyroidism, GL aids in restoring normalcy (Table 1).

Table 1. Effects of UVB irradiation and glycyrrhizin on body and thyroid weight and thyroid hormone levels in control and experimental animals

Variables	I	II	III
Body weight (gm)	29±0.70	22.94±0.95**	25.50±0.57**
Thyroid weight (gm)	0.092±0.003	0.068±0.002**	0.083±0.004*
T3 (ng/dL)	1.07±0.09	2.02±0.06***	1.41±0.07**
T4 (ng/dL)	7.69±0.46	12.71±0.40***	9.43±0.62**
TSH (µIU/mL)	4.50±0.13	1.60±0.12***	2.90±0.14**

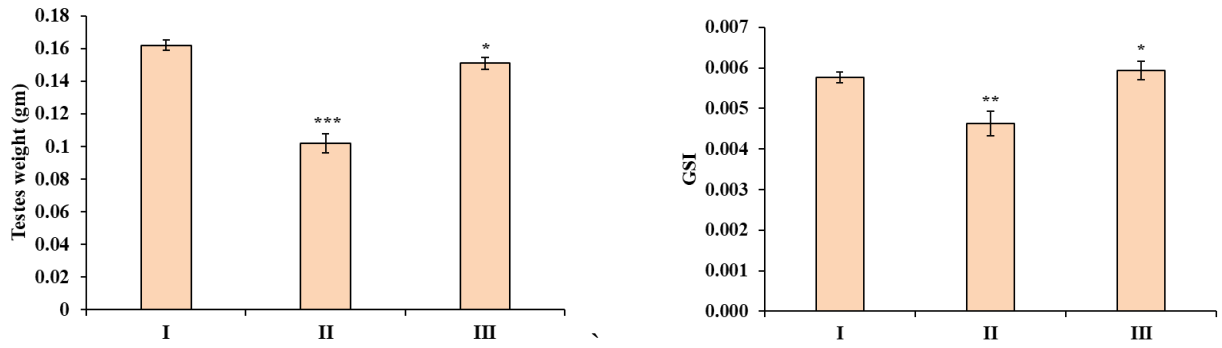
The data are the means ± SEs; n = 6. Differences between the control group and the other groups were considered significant at p <0.001 (***), p <0.01 (**), and p <0.05 (*). P values were obtained from one-way ANOVA, followed by Tukey's test for multiple comparisons.

Effect of UVB radiation-induced hyperthyroidism on the testis

Testis weight and gonadal somatic index (GSI)

The testicle weight in the UVB radiation-induced hyperthyroidism group was considerably lower than that in the control group (***p<0.001). However, in the group receiving glycyrrhizin, the testicle weight considerably increased (*p<0.05) (Fig. 2). A).

In addition, the GSI in the UVB radiation-induced hyperthyroidism group decreased considerably (**p<0.01). However, in the group receiving glycyrrhizin, the GSI considerably increased (*p<0.05) (Fig. 2. B).



(A) **(B)**

Fig 2. Effect of UVB irradiation and glycyrrhizin administration on testicle weight (A) and the gonadal-somatic index (B). The values are presented as the means ± SEs (n = 6). Significant difference from the control group (*p<0.05, **p<0.01, ***p<0.001)

Plasma Levels of LH and FSH and Testosterone and Sperm Counts

The serum follicle stimulating hormone (**p<0.01), luteinizing hormone (***p<0.001) and testosterone (***p<0.001) levels were significantly lower in the UVB radiation-induced hyperthyroidism group than in the control group and increased significantly (*p<0.05; **p<0.01) after the administration of glycyrrhizin compared to those in the UVB radiation-induced hyperthyroidism group (Table 2).

The sperm count decreased significantly (***p<0.001) in the UVB radiation-induced hyperthyroidism group compared to that in the control group and increased significantly (**p<0.01) in the glycyrrhizin administration group compared to that in the UVB radiation-induced hyperthyroidism group (Table 2).

Table 2. Effects of UVB radiation-induced hyperthyroidism and glycyrrhizin on FSH, LH and testosterone levels and sperm counts in control and experimental animals

Variables	I	II	III
FSH (mIU/mL)	12.61±0.42	8.81±0.43**	9.01±0.46*
LH (mIU/mL)	13.13±0.40	8.30±0.33***	10.48±0.57**
Testosterone (ng/mL)	8.12±0.38	4.56±0.20***	6.67±0.20**
Sperm Count (millions/mL)	36.56±1.60	15.05±1.69***	26.68±1.47**

The data are the means ± SEs; n = 6. Differences between the control group and the other groups were considered significant at p <0.001 (***), p <0.01 (**), and p <0.05 (*). P values were obtained from one-way ANOVA, followed by Tukey's test for multiple comparisons.

Effects of UVB Radiation and GL on Enzymatic and Nonenzymatic Antioxidants

Compared with that in the control group, the amount of LPO significantly increased (**p<0.01), but the level significantly decreased when GL was administered to the UVB radiation-induced hyperthyroidism group (*p<0.05). GSH levels were lower (**p<0.01) in the UVB radiation-induced hyperthyroidism group than in the control group, but GSH levels increased after GL intake (**p<0.01). The activity of GR was significantly greater (**p<0.01) in the UVB radiation-induced hyperthyroidism group than in the control group and decreased in the GL-treated group (Table 3).

SOD and CAT levels decreased significantly (**p<0.01; ***p<0.001) in the UVB radiation-induced hyperthyroidism group, while SOD and CAT levels significantly increased (*p<0.05; **p<0.01) after GL treatment (Table 3).

H2O2 and NO levels were significantly greater (**p<0.01) in the UVB radiation-induced hyperthyroidism group than in the control group, while H2O2 and NO levels decreased significantly (*p<0.05) after GL treatment (Table 3).

Table 3. Effects of UVB radiation-induced hyperthyroidism and glycyrrhizin on enzymatic and nonenzymatic antioxidants

Variables	**I**	**II**	**III**
LPO (n mol/mg protein)	20.64±0.69	24.16±0.50**	20.36±0.50*
CAT (U/mg protein)	76.08±2.29	31.27±1.86***	58.95±3.69**
H2O2 Conc. (µM/mg protein)	0.28±0.04	0.62±0.05**	0.40±0.05*
SOD (U/mg protein)	359.96±10.43	271.30±8.17**	339.99±12.27*
NO (µm/mL/mg protein)	2.82±0.83	6.97±0.43**	5.87±0.29*
GSH (µM/mg protein)	2.38±0.18	0.79±0.20**	1.49±0.21**
GR (U/mg protein)	188.84±7.30	390.45±12.66**	299.74±8.65*

The data are presented as the means ± SEMs; n = 6. Differences between the control group and the other groups were considered significant at p<0.001 (***), p<0.01 (**), and p<0.05 (*). P values were obtained from one-way ANOVA, followed by Tukey's test for multiple comparisons. LPO, lipid peroxidation; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; H2O2, hydrogen peroxide; GR, glutathione reductase; NO, nitric oxide

Histological analysis

Histopathological investigations revealed a wide range of tubular architecture damage in UVB radiation-induced hyperthyroidism mice compared to control mice. The cells in the UVB radiation-induced hyperthyroidism group were elongated. Injured tubules showed signs of necrotic cells, pyketic nuclei, karyorrhexis, cytoplasmic degranulation, and cytoplasmic vacuolation. In addition to cytoplasmic debris, exfoliation, and a very small number of degenerating cells in some tubules, there was a significant amount of intertubular edema throughout the testicular tissue. Both thickening and tubular adhesion of the basement membrane were common. There were also a few tubules and massive multinucleated cells visible. The germinal epithelium was widely disorganized, and spermatogenesis had completely stopped. On the other hand, in animals receiving GL, the tubular structure seemed to return quickly to a normal structure with a large population of spermatogenic cells after undergoing a severe degenerative phase of hyperthyroidism caused by UVB radiation (Figure 3).

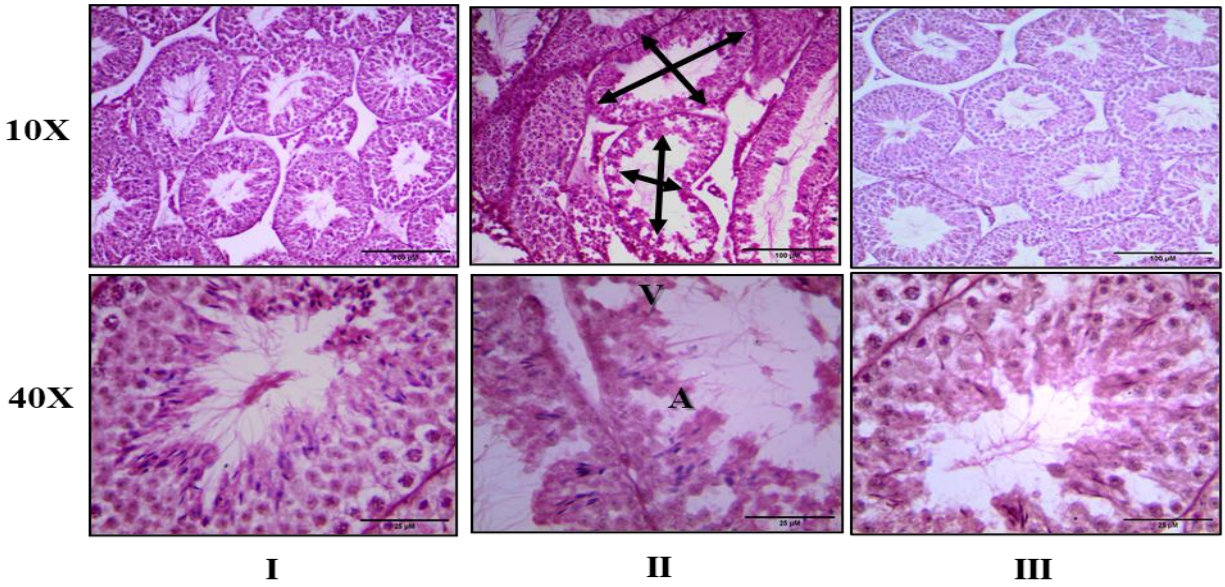


Fig. 3. Modulation of UVB radiation induced hyperthyroidism histological changes in testes of *Swiss albino* mice by glycyrrhizin. Photomicrograph I showing the normal architecture of the testis. Photomicrograph II showing the disrupted germinal epithelium, empty tubules (large arrow), intertubular edema, pyketic nuclei (small arrow), vacuoles in the germinal epithelium (V), and atrophy (A) with a depleted germ cell population. Photomicrograph III shows better testicular architecture in the GL-treated group. Photograph showing H&E staining (100X and 400X).

Alterations in the central and peripheral tubular diameters were observed in the UVB radiation-induced hyperthyroidism group; these differences were significant (**p<0.01) compared to those in the control group. However, the administration of GL significantly decreased (*p<0.05) the tubular diameter (Table 4).

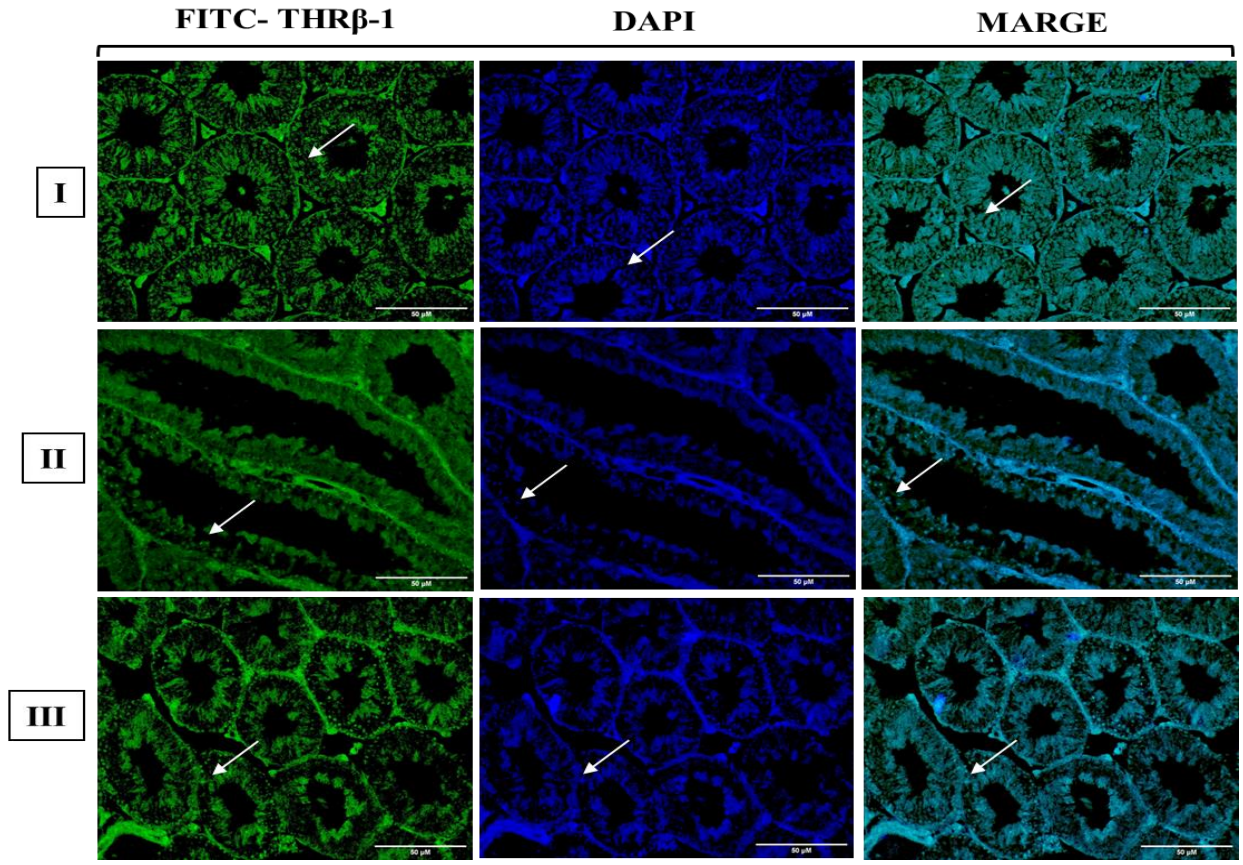
Table 4. Effects of UVB radiation-induced hyperthyroidism and glycyrrhizin on the peripheral and central diameters of the seminiferous tubules

Variables	**I**	**II**	**III**
Peripheral diameter (µM)	155.12±7.04	205.74±4.38**	175.65±6.39*
Central diameter (µM)	161.25±6.40	197.84±2.97**	170.62±5.41*

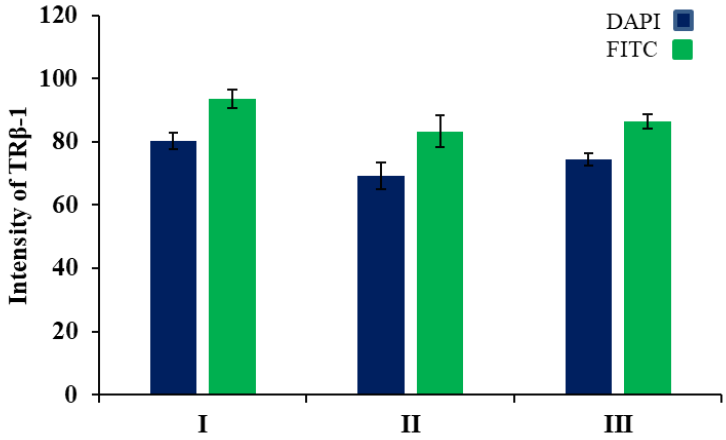
The data are the means ± SEs; n = 6. Differences between the control group and the other groups were considered significant at p <0.001 (***), p <0.01 (**), and p <0.05 (*). P values were obtained from one-way ANOVA, followed by Tukey's test for multiple comparisons.

Immunofluorescence Analysis of THRβ-1 Expression

Immunofluorescence staining was utilized to quantify the expression of THRβ-1 in the seminiferous tubules of the testis. An immunofluorescence photomicrograph of the seminiferous tubules expressing THRβ-1 is shown in Fig. 4. The location of the nucleus was ascertained using DAPI staining. The combined images demonstrate the colocalization of FITC and DAPI dual staining. The seminiferous tubules of the control group exhibited increased THRβ-1 expression. Conversely, the testis sections from the UVB radiation-induced hyperthyroidism group exhibited a reduction in THRβ-1 expression in the seminiferous tubules. Compared to the group that experienced UVB radiation-induced hyperthyroidism, the administrative group exhibited higher levels of THRβ-1 expression.



(A)



(B)

Fig. 4. Immunofluorescence localization of THRβ-1. TS of the testes of the control group, the UVB radiation-induced hyperthyroidism group and the UVB+GL coadministrative group. (A) Photograph showing DAPI and FITC staining, where the arrow indicates the expression of the THRβ-1 antibody in the testis (200X). (B) Graph of the fluorescence intensity in the testis, where the values represent the means ± SEs (n= 6).

Immunofluorescence Expression of Caspase-3

Immunofluorescence staining was used to quantify Casp-3 expression in the seminiferous tubules of the testis. An immunofluorescence photomicrograph of the seminiferous tubules expressing Casp-3 is shown in Fig. 5. DAPI staining was used to determine the location of the nucleus. The combined images demonstrate the colocalization of FITC and DAPI dual staining. Casp-3 expression was lower in the seminiferous tubules of the control group than in those of the control group. Conversely, the seminiferous tubules of the testes of the UVB radiation-induced hyperthyroidism group exhibited elevated Casp-3 expression. Casp-3 expression was lower in the treatment group than in the UVB radiation-induced hyperthyroidism group.

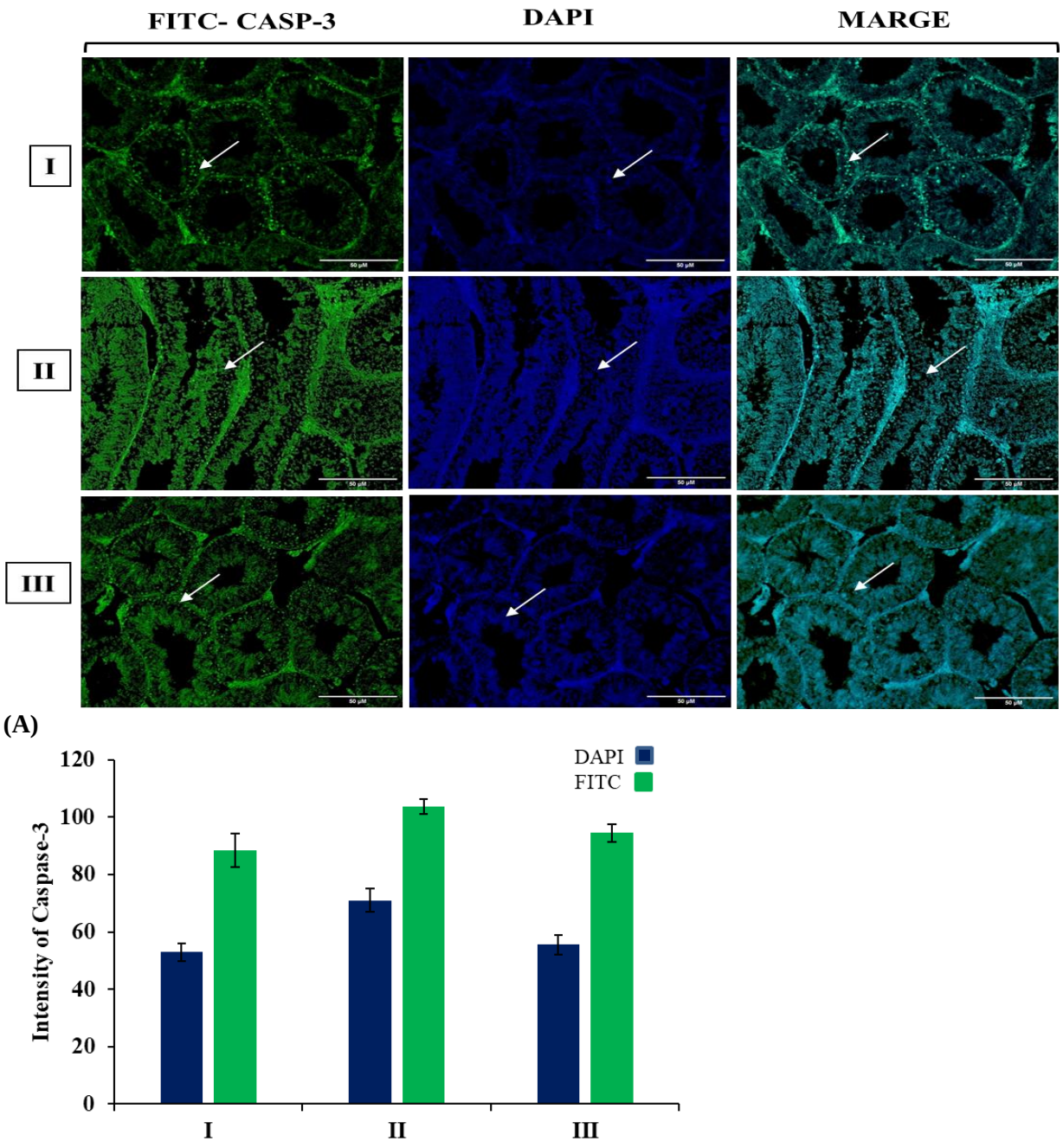
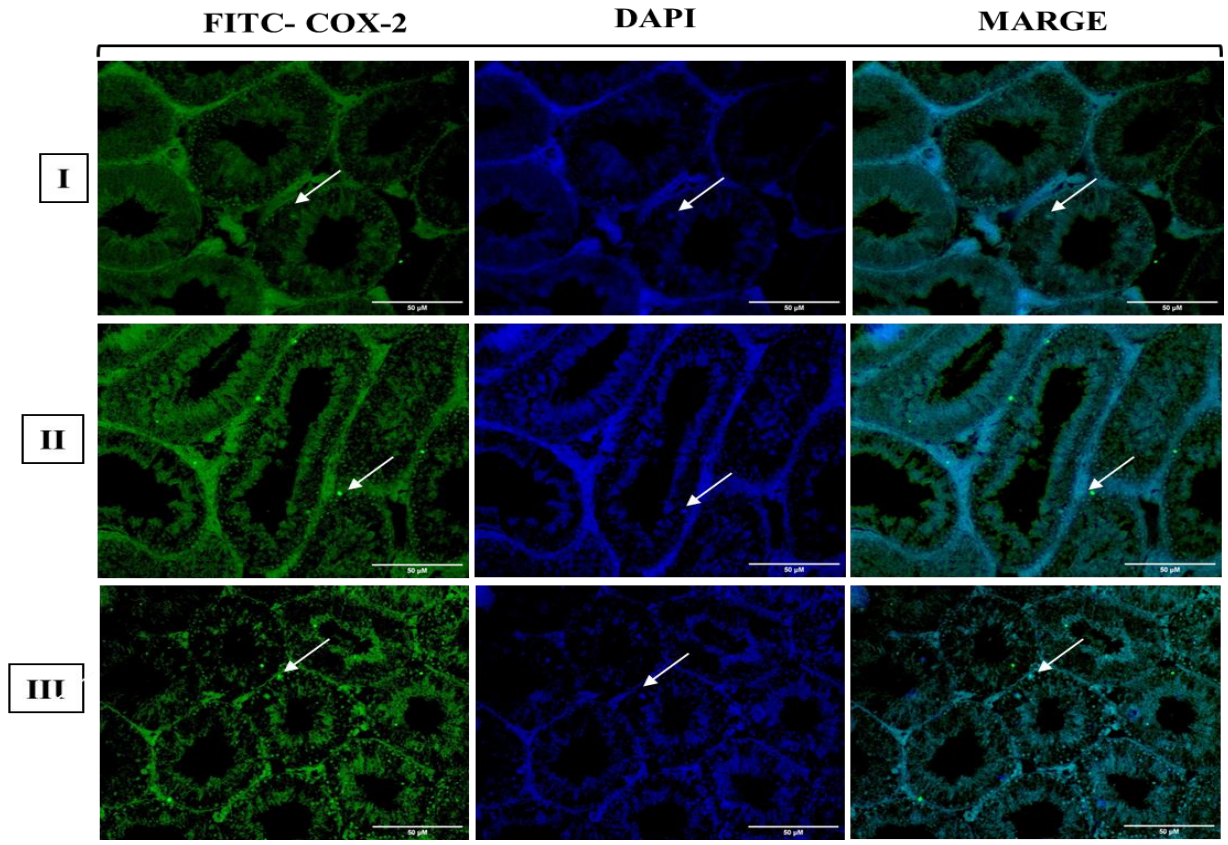


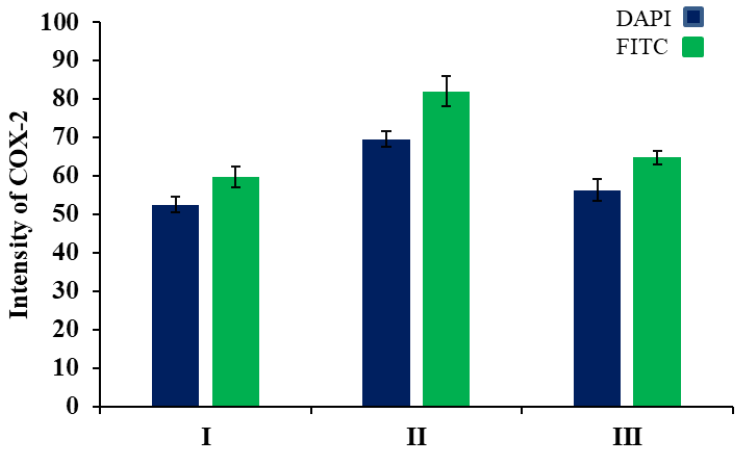
Fig. 5. Immunofluorescence localization of Casp-3. TS of the testes of the control group, the UVB radiation-induced hyperthyroidism group and the UVB+GL coadministrative group. (A) Photograph showing DAPI and FITC staining where the arrow indicates the expression of the Casp-3 antibody in the testis (200X). (B) Graph of the fluorescence intensity in the testis, where the values represent the means ± SEs (n= 6).

Immunofluorescence Expression of COX-2

Immunofluorescence staining was used to quantify COX-2 expression in the seminiferous tubules of the testes. The locations of COX-2-expressing seminiferous tubules are shown in an immunofluorescent photomicrograph in Figure 6. One method for locating the nucleus is to use DAPI staining. The combination photos display the colocalization of FITC and DAPI dual staining. Reduced expression of COX-2 was observed in the seminiferous tubules of the control group. However, greater COX-2 expression in the seminiferous tubules was detected in the testes of the UVB radiation-induced hyperthyroidism group. Compared with those in the UVB radiation-induced hyperthyroidism group, the COX-2 expression levels in the control group were lower.



(A)

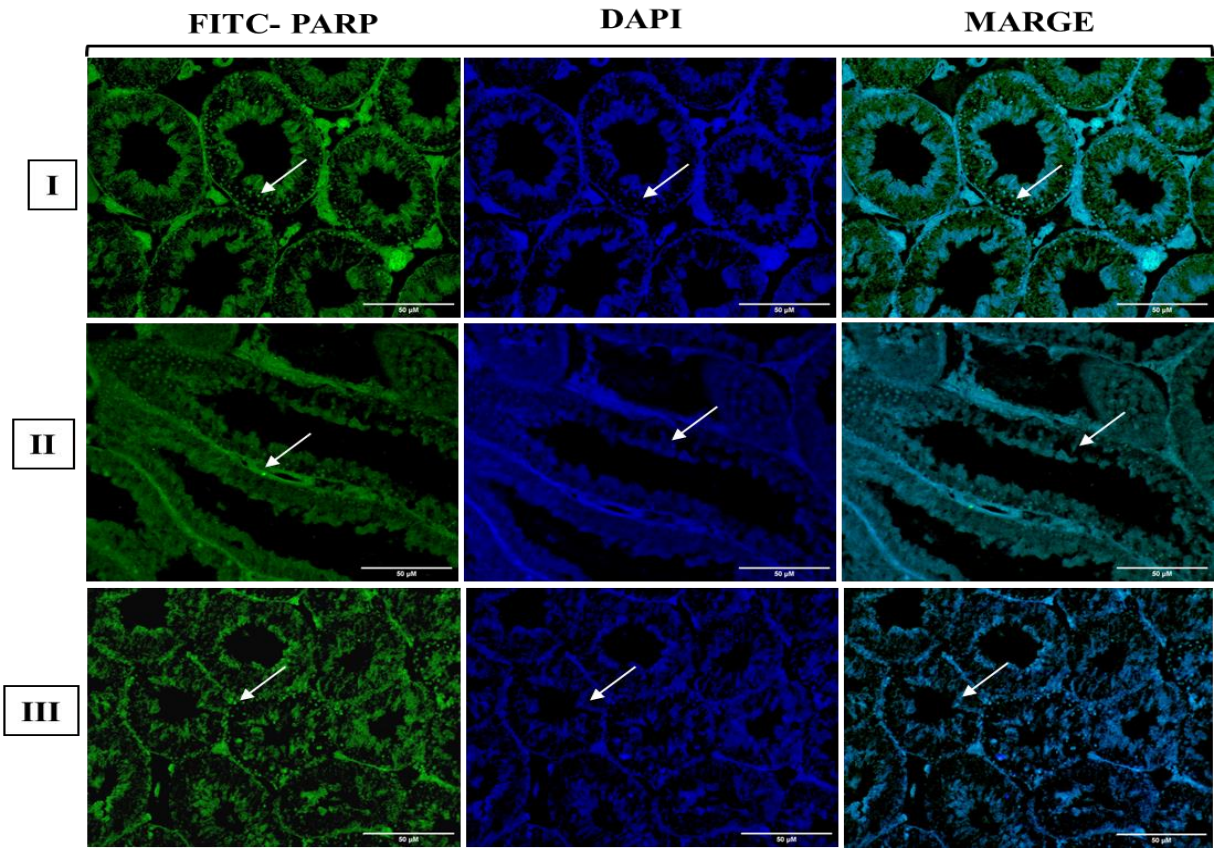


(B)

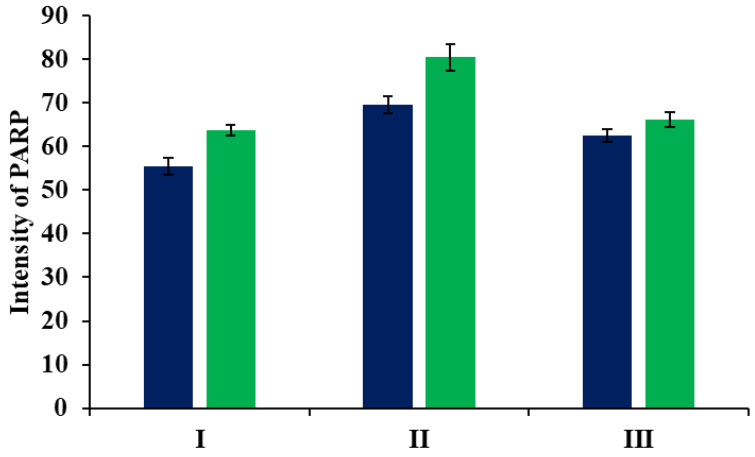
Fig. 6. Immunofluorescence localization of COX-2. TS of the testes of the control group, the UVB radiation-induced hyperthyroidism group and the UVB+GL coadministrative group. (A) Photograph showing DAPI and FITC staining, where the arrow indicates the expression of COX-2 in the testis (200X). (B) Graph of the fluorescence intensity in the testis, where the values represent the means ± SEs (n= 6).

Immunofluorescence Analysis of PARP Expression

The expression of PARP in the seminiferous tubules of the testis was quantified via immunofluorescence staining. An immunofluorescence photomicrograph showing the location of PARP expressed in the seminiferous tubules is shown in Fig. 7. The location of the nucleus was established through DAPI staining. The combined images display the colocalization of FITC and DAPI dual staining. The seminiferous tubules of the control group exhibited reduced PARP expression. Conversely, the seminiferous tubules of the testes of the UVB radiation-induced hyperthyroidism group exhibited elevated PARP expression. Compared to the group that experienced UVB radiation-induced hyperthyroidism, the administrative group had reduced PARP expression.



(A)



(B)

Fig. 7. Immunofluorescence localization of PARP. TS of the testes of the control group, the UVB radiation-induced hyperthyroidism group and the UVB+GL coadministrative group. (A) Photograph showing DAPI and FITC staining, where the arrow indicates the expression of the PARP antibody in the testis (200X). (B) Graph of the fluorescence intensity in the testis, where the values represent the means ± SEs (n= 6).

Discussion

In recent decades, the multivariate effects of thyroid hormones on male reproductive function have been extensively studied, aiming to better understand the relationship between these hormones and male infertility (**Krajewska-Kulak and Sengupta, 2013**), and various studies have suggested that reproductive failure, altered semen quality, subnormal sperm count, and motility are closely associated with hypo- and hyperthyroid states (**Jannini *et al*., 1995**). Thyroid hormones are crucial physiological regulators, as deficiency leads to decreased cellular activity. A dual regulatory role of thyroid hormone on Leydig cell functions has been proposed (**Sharpe, 2010; La Vignera and Vita, 2018**). However, information on its role in regulating adult testis physiological and biochemical functions, particularly antioxidant defenses, is inadequate.

Our study revealed that UVB radiation is responsible for the induction of hyperthyroidism in male mice. A significant increase (***p<0.001) in the T3 and T4 concentrations and a decrease (***p<0.001) in the TSH concentration, as well as a significant decrease (**p<0.01) in the body and thyroid weights of male mice, confirmed the induction of hyperthyroidism in male mice (**Table 1**). UVB radiation affects the function of the thyroid gland and induces hyperthyroidism in male mice, as shown in our previous reports (**Shakyawal and Mahobiya, 2023**). In the present study, the testis weight (***p<0.001) and gonadosomatic index (**p<0.01) were significantly lower in the UVB radiation-induced hyperthyroidism group than in the control group (**Figure 2. A. B**). **Choudhury *et al*. (2003**) reported that hyperthyroidism induced by 20 µg of T3 per body weight in PTU-treated rats significantly decreased the testis weight and gonadosomatic index. T3 deficiency leads to abnormal testicular growth, decreased testis weight, and steroidogenesis (**Maran, 2003**). Altered thyroid hormones lead to decreased GSI, indicating altered reproductive organ growth, possibly due to decreased testis weight and body weight. In the present study, glycyrrhizin administration to mice with UVB radiation-induced hyperthyroidism significantly decreased the testis weight and GSI. A previous study revealed that treatment with glycyrrhizin (25 mg/kg b.w.) significantly increased the testis weight and GSI in Wistar rats with diazinon-induced toxicity (**Karimani *et al*., 2019**). Some studies have also reported that aqueous liquorice extract (50 mg/kg b.w.) increases the weight of the testis in response to carbendazim-induced toxicity in the testis of albino rats (**Sakr and Shalaby, 2014**).

Normal spermatogenesis relies on testosterone secretion and the gonadotropic hormonal action of LH and FSH, with LH stimulating Leydig cells and FSH acting on seminiferous tubules, which are essential for initiation and maintenance (**Spaliviero *et al*., 2004; Rago & Salacone, 2008**). In the present study, the serum concentrations of FSH (**p<0.01), LH (***p<0.001) and testosterone (***p<0.001) decreased significantly in UVB radiation-induced hyperthyroidism mice compared with those in control mice and increased after the administration of glycyrrhizin (**Table 2**). Rijntjes *et al*., in their study on rats, concluded that hyperthyroidism delays Leydig cell development and adversely affects spermatogenesis. Hyperthyroidism inhibits Leydig cell activity, reducing testosterone synthesis or antiandrogenic activity. FSH and LH from the anterior pituitary regulate testosterone synthesis in males. The authors reported that induced hyperthyroidism significantly decreased the concentrations of FSH, LH and testosterone in Wistar rats (**Rijntjes *et al*., 2008**). Hyperthyroidism leads to elevated T4 levels, impaired LH and follicle-stimulating hormone responsiveness, and altered endocrine profiles, affecting testicular function, morphology, spermatogenesis, stunted development, and motility (**Alahmar *et al*., 2019**). Thyroid hormones play a crucial role in the development and maturation of Leydig and Sertoli cells, which are essential for steroidogenesis and spermatogenesis in the male reproductive system (**Maran, 2003**). The serum concentration of testosterone leads to the development and maturation of spermatozoa. In the present study, the sperm count (***p<0.001) decreased significantly in the UVB radiation-induced hyperthyroidism group compared to the control group and increased after glycyrrhizin administration (**Table 2**). The decrease in sperm count is probably due to the altered effects of thyroid hormones on Sertoli and Leydig cells. Experimentally induced hyperthyroidism in male rats causes a significant reduction in the number of sperm and motility (**Asker *et al*., 2015**). The decreased spermatozoa count is probably due to increased spermatozoa death due to undernourishment caused by damaged interstitial cells (**Chattopadhyay *et al*., 2010**). Thyroid hormones impact male reproductive physiology by decreasing testosterone and spermatozoa, leading to abnormalities and poor Sertoli cell development, resulting in infertility in males (**Singh *et al*., 2011**). In contrast, the extract of *Glycyrrhiza glabra* (100 mg/kg b.w.) improved testicular hormone levels and sperm count in response to ochratoxin A-induced testis damage in mature rats (**Malekinejad *et al*., 2011**).

Testicular oxidative stress is caused by thyroid hormone regulation of oxygen use and mitochondrial energy synthesis. The production of ROS is accelerated, and antioxidant activity is decreased when thyroid hormone levels are altered (**Choudhury *et al*., 2003**). Nonradical oxygen species called reactive oxygen species (ROS) can be harmful to cells if they surpass their antioxidant threshold, resulting in oxidative stress and defense system growth (**Venditti and Meo, 2006**). In the present study, compared with the control, UVB radiation-induced hyperthyroidism significantly increased the MDA concentration (**p<0.01) and increased the activity of H2O2 and NO (**p<0.01) (**Table 3**). A prior study indicated that the presence of oxidative stress in rat testes under hyperthyroid conditions was linked to increased levels of mitochondrial hydrogen peroxide and lipid peroxidation (**Sahoo *et al*., 2005**). Moreover, research has shown that NO signaling plays a major role in the pathophysiology of inflammation (**Napoli *et al*., 2013**). In the present study, the enzymatic activity of SOD (**p<0.01) and catalase (***p<0.001) decreased significantly in the testes of UVB radiation-induced hyperthyroidism mice compared with those of control mice (**Table 3**). GR activity increased (**p<0.01) significantly, while GSH activity decreased (**p<0.01) significantly in the testes of hyperthyroidism mice

(**Table 3**). Decreased antioxidant activity is a sign that the antioxidant mechanism is affected by thyroid hormone shortage, which makes it difficult for the endogenous antioxidant system to combat oxidative stress. Previous research has shown that in hyperthyroidism models, the activities of both enzymatic and nonenzymatic antioxidants decrease, leading to an increase in oxidative stress (**Ramadan *et al*., 2021**). Because of increased lipid peroxidation, hypermetabolic activity, consumption during lipid peroxidation modulation, and decreased enzyme activity, which results in decreased production, hyperthyroidism may cause lower GSH levels (**Asayama and Kato, 1990; Bayda and Meral, 2005**). Chattopadhyay *et al*. (2007) and Babu *et al*. (2011) reported a decrease in superoxide dismutase (SOD) levels in hyperthyroidism rats, as overproduction of ROS suppresses SOD activity (**Babu *et al*., 2011; Chattopadhyay *et al*., 2007**). The results of the present study are consistent with those of earlier studies, which indicated that hyperthyroidism causes oxidative damage to the testicles. In contrast, glycyrrhizin therapy increased the MDA and GSH levels in the AR mice, indicating its antioxidant qualities. Preventing reactive intermediate conjugation to GSH mitigated oxidative damage. Glycyrrhizin has been shown to alleviate oxidative stress by rectifying the antioxidant system, as indicated by the increase in enzyme activity (SOD and catalase) following treatment with glycyrrhizin and Lycopene (**Li *et al*., 2011**).

The reproductive physiology of males is altered by impaired thyroid hormones and structural abnormalities in Leydig and Sertoli cells within the seminiferous tubules, resulting in abnormalities in testicular structures (**Alahmar *et al*., 2019**). The histological analysis of the testes from the hyperthyroid group in this study revealed that the normally round shape of the seminiferous tubules had disappeared, appearing as irregular structures with corrugations in the basement membrane. Additionally, there was a notable decrease in the height of the germinal epithelium in the tubules, as well as prominent Sertoli cells, some with no sperm, exfoliated cells, giant cells, wide interstitial spaces, and thick blood vessels (**Figure 3**). Abo-Elnour and El-Deeb found that rats injected intraperitoneally with T4 (40 μg/kg for 28 days) lost their seminiferous tubules, causing mitotic arrest, apoptosis, and acidophilic material deposition. This could be due to oxidative stress from hyperthyroidism (**Abo-Elnour and El-Deeb, 2012**). Another study by Asker *et al*. revealed severe spermatogenic arrest in many tubules after T4 (300 μg/kg for 3 weeks) was injected into rats (**Asker *et al*., 2015**). In our investigation, glycyrrhizin in the testis protected against these abnormalities. The testis displayed morphometric abnormalities, suppression of spermatogenesis, and histological modifications. According to a study, mature Wistar rats treated with liquorice (glycyrrhizin; 50 mg/kg b.w.) had testes with few injured cells, intact tubules, and normal spermatogenic development, with normal spermatozoa filling the lumen against toxicity caused by 100 mg/kg b.w. carbendazim (**Sakr and Shalaby, 2014**). Licorice, also known as *Glycyrrhiza uralensis Fisch*., has been reported to promote cell proliferation and detoxification in C57BL/6 N mouse testes at doses of 0.2, 2, and 20 μM for 72 hours (**Wang *et al*., 2016; Noh *et al*., 2020**).

THRβ-1, a key hormone in developing Sertoli cells, plays a crucial role in spermatogenesis and fertility, and decreased expression of this hormone could impact male reproductive physiology and fertility (**Jannini *et al*., 2000; Rao *et al*., 2003; Wagner *et al*., 2008**). The present study revealed that the immunolocalization and fluorescence of THRβ-1 on Sertoli and Leydig cells decreased significantly (**$p<0.01$) in hyperthyroid mice compared with those in control mice (**Figure 4**). The current study revealed that low thyroid hormone levels in the testis result in altered testosterone synthesis, reduced spermatogenesis, and decreased expression of THRβ-1 in the seminiferous tubules. Furthermore, research indicates that thyroid hormone receptor expression in the testis, reproductive tract, and accessory organs is markedly reduced by hyperthyroidism, particularly in Sertoli cells, which are important for fertility (**Kumar *et al*., 2014; Elbers *et al*., 2016**).

According to previous studies, immunofluorescence detection of Caspase-3 is a reliable method for identifying apoptosis before any morphological cellular changes occur (**Gown and Willingham, 2002**). In the present study, the biochemical status of histopathological modifications in hyperthyroidism mice was investigated, and caspase-3 immunoreactivity was greater than that in control mice (**Figure 5**), indicating an increased state of cellular death and induced apoptosis. Caspase-3 is a crucial executional caspase responsible for cleaving intracellular compartments during apoptosis (**Kopeina *et al*., 2018**). Previous studies have shown that apoptosis is crucial in various pathologic conditions, and patients with thyrotoxicosis exhibit morphological changes in their livers due to necrotic cell death (**Bhattacharyya and Wiles, 1997**). Hassan *et al*. reported that hyperthyroidism significantly influenced caspase-3 activation and induced apoptosis in epithelial cells of the tongue (**Hassan and Zahran, 2019**).

Inflammatory damage to the male genital tract leads to increased reactive oxygen species (ROS), with pathogenic bacterial strains exacerbating this inflammatory response (**Dutta *et al*., 2021**). In the present study, the fluorescence of COX-2 was significantly greater (**$p<0.01$) in the hyperthyroid group than in the control group (**Figure 6).** According to earlier research, inflammation in Leydig cells causes apoptosis, decreased

antioxidant enzyme concentrations, worsened mitochondrial membrane potential, and enhanced COX-2 synthesis (**Azenabor *et al*., 2015; Ko *et al*., 2014**).
A previous study explored the role of poly(ADP-ribosyl)ation of proteins, which is catalyzed by poly(ADP-ribose) polymerase (PARP), in rat testis spermatogenesis, indicating its potential to regulate germ cell chromatin organization and DNA metabolism events (**Dantzer *et al*., 2006; Mennella *et al*., 2003**). In the present study, compared with control mice, hyperthyroid mice showed significantly greater (**p<0.01) florescence in the seminiferous tubules, indicating DNA damage (**Figure 7**). Earlier reports showed that T3 treatment (10 µg/100 gm b.w.) increased poly(ADP-ribose) polymerase activity in hyperthyroid rats, with increased turnover of both poly(ADP-ribose) polymerase and poly(ADP-ribose) glycohydrolase compared to that in euthyroid animals (**Faraone-Mennella *et al*., 2009**). In addition, Chakraborty *et al*. reported that excessive iodine intake increased the expression of proapoptotic markers (Bid, Bax) and p53, along with other apoptotic modulators and effectors, such as cytochrome c, cleaved PARP, caspase-3, and caspase-9, in male albino rats (**Chakraborty *et al*., 2020**).
In contrast, the administration of glycyrrhizin significantly decreased the florescence of caspase-3, COX-2 and PARP compared to that in the hyperthyroid group (**Figure 5,6,7**). Previous studies revealed that the increase in the apoptotic index was reversed by glycyrrhizin acid (glycyrrhizin) (100 mg/kg b.w.) in rats with hepatotoxicity induced by titanium dioxide nanoparticles (**Orazizadeh *et al*., 2020**). Xiao *et al*. reported that 18β-glycyrrhizin (75 mg/kg b.w.) ameliorates acute *PA*-induced liver injury through the inhibition of macrophage inflammatory proteins (**Xiao *et al*., 2010**). Ju *et al*. reported that liquorice and its active compound glycyrrhizic acid (glycyrrhizin) ameliorate cisplatin-induced nephrotoxicity by inhibiting caspase-3 and PARP activity (**Ju *et al*., 2017**).
A previous study revealed that thyroid hormones significantly influence male gonad development, and changes in these hormones can impact testicular function (**Wajner *et al*., 2009; Flood *et al*., 2013**). The results of the current study demonstrate the critical role that thyroid hormones play in the development of male reproduction, with hyperthyroidism having an impact on DNA damage, testis damage, testosterone synthesis, and the semen profile. UVB radiation-induced hyperthyroidism impacts the ability to reproduce. It is associated with oxidative stress and impaired antioxidant function. Moreover, glycyrrhizin administration dramatically reduces the risk of hyperthyroidism. These findings support the results of the present study and demonstrate how glycyrrhizin prevents apoptosis and inflammation in response to UVB radiation-induced hyperthyroidism, which results in testicular failure.

Acknowledgment

This research work was financially supported by UGC NON-NET FELLOWSHIP for booming out this research work efficaciously. The authors acknowledge to DST-FIST sponsored Department of Zoology, School of Biological Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar, M.P., India, for providing infrastructure facilities.

Data Availability
Data will be provided on request.

Conflict of interest statement
The authors declare that there is no conflict of interest.

Authors' contributions
Payal Mahobiya designed the experiment plan. Shashank Shakyawal managed the experimental animals, performed the treatment and completed data analysis and wrote the manuscript. Gayatri Rai and Zaved Ahmad contributed to the editing and completion of the manuscript. All the authors read and approved the final manuscript.

References

1. Abo-Elnour, R.K. and El-Deeb, D.F., 2012. A histological study on the effect of experimentally induced hyperthyroidism on adult albino rat testis. *Egyptian Journal of Histology*, *35*(4), pp.862-871.

2. Akindele, A.J., Adeneye, A.A., Salau, O.S., Sofidiya, M.O. and Benebo, A.S., 2014. Dose and time-dependent sub-chronic toxicity study of hydroethanolic leaf extract of Flabellaria paniculata Cav.(Malpighiaceae) in rodents. *Frontiers in pharmacology*, *5*, p.78.
3. Alahmar, A., Dutta, S. and Sengupta, P., 2019. Thyroid hormones in male reproduction and infertility. *Asian Pacific Journal of Reproduction*, *8*(5), pp.203-210.
4. Asayama, K. and Kato, K., 1990. Oxidative muscular injury and its relevance to hyperthyroidism. *Free Radical Biology and Medicine*, *8*(3), pp.293-303.
5. Asker, M.E., Hassan, W.A. and El-Kashlan, A.M., 2015. Experimentally induced hyperthyroidism influences oxidant and antioxidant status and impairs male gonadal functions in adult rats. *Andrologia*, *47*(6), pp.644-654.
6. Azenabor, A., Ekun, A.O. and Akinloye, O., 2015. Impact of inflammation on male reproductive tract. *Journal of reproduction & infertility*, *16*(3), p.123.
7. Babu, K., Jayaraaj, I.A. and Prabhakar, J., 2011. Effect of Abnormal thyroid hormone changes in lipid peroxidation and Antioxidant imbalance in Hypothyroid and Hyperthyroid patients. *Int J Biol Med Res*, *2*(4), pp.1122-1126.
8. Baghel, K. and Srivastava, R., 2021. Photoperiod dependent expression of estrogen receptor alpha in testes of Japanese quail: Involvement of Withania somnifera in apoptosis amelioration. *Biochemical and Biophysical Research Communications*, *534*, pp.957-965.
9. Baydas, B. and Meral, I., 2005. Effects of melatonin on lipid peroxidation and anti-oxidant enzyme activity in rats with experimentally induced hyperthyroidism. *Clinical and experimental pharmacology and physiology*, *32*(7), pp.541-544.
10. Beauchamp, C. and Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), pp.276-287.
11. Bergmeyer HU. Methods of enzymatic analysis. Weinheim, Germany: Verlag Chemie 1983. 3, 3 273-286.
12. Bhattacharyya, A. and Wiles, P.G., 1997. Thyrotoxic crisis presenting as acute abdomen. *Journal of the Royal Society of Medicine*, *90*(12), pp.681-682.
13. Cadet, J., Douki, T. and Ravanat, J.L., 2015. Oxidatively generated damage to cellular DNA by UVB and UVA radiation. *Photochemistry and photobiology*, *91*(1), pp.140-155.
14. Carlberg, I.N.C.E.R. and Mannervik, B.E.N.G.T., 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *Journal of biological chemistry*, *250*(14), pp.5475-5480.
15. Chakraborty, A., Singh, V., Singh, K. and Rajender, S., 2020. Excess iodine impairs spermatogenesis by inducing oxidative stress and perturbing the blood testis barrier. *Reproductive Toxicology*, *96*, pp.128-140.
16. Chattopadhyay, S., Choudhury, S., Roy, A., Chainy, G.B. and Samanta, L., 2010. T3 fails to restore mitochondrial thiol redox status altered by experimental hypothyroidism in rat testis. *General and Comparative Endocrinology*, *169*(1), pp.39-47.
17. Chattopadhyay, S., Sahoo, D.K., Subudhi, U. and Chainy, G.B.N., 2007. Differential expression profiles of antioxidant enzymes and glutathione redox status in hyperthyroid rats: a temporal analysis. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *146*(3), pp.383-391.
18. Choudhury, S., Chainy, G.B.N. and Mishro, M.M., 2003. Experimentally induced hypo-and hyper-thyroidism influence on the antioxidant defence system in adult rat testis. *Andrologia*, *35*(3), pp.131-140.
19. Dantzer, F., Mark, M., Quenet, D., Scherthan, H., Huber, A., Liebe, B., Monaco, L., Chicheportiche, A., Sassone-Corsi, P., de Murcia, G. and Ménissier-de Murcia, J., 2006. Poly (ADP-ribose) polymerase-2 contributes to the fidelity of male meiosis I and spermiogenesis. *Proceedings of the National Academy of Sciences*, *103*(40), pp.14854-14859.
20. Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R. and Sadeghi, M.R., 2018. Reactive oxygen species and male reproductive hormones. *Reproductive Biology and Endocrinology*, *16*, pp.1-14.
21. Dasdag, S., Akdag, M.Z., Erdal, M.E., Erdal, N., Ay, O.I., Ay, M.E., Yilmaz, S.G., Tasdelen, B. and Yegin, K., 2015. Effects of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on microRNA expression in brain tissue. *International journal of radiation biology*, *91*(7), pp.555-561.

22. Dowlath, M.J.H., Karuppannan, S.K., Sinha, P., Dowlath, N.S., Arunachalam, K.D., Ravindran, B., Chang, S.W., Nguyen-Tri, P. and Nguyen, D.D., 2021. Effects of radiation and role of plants in radioprotection: A critical review. *Science of the Total Environment*, *779*, p.146431.
23. Dutta, S., Sengupta, P., Slama, P. and Roychoudhury, S., 2021. Oxidative stress, testicular inflammatory pathways, and male reproduction. *International journal of molecular sciences*, *22*(18), p.10043.
24. Elbers, L.P., Moran, C., Gerdes, V.E., van Zaane, B., Meijers, J.C., Endert, E., Lyons, G., Chatterjee, K., Bisschop, P.H. and Fliers, E., 2016. The hypercoagulable state in hyperthyroidism is mediated via the thyroid hormone β receptor pathway. *European journal of endocrinology*, *174*(6), pp.755-762.
25. Faraone-Mennella, M.R., Ferone, A., Marino, L., Cardone, A., Comitato, R., Venditti, P., Di Meo, S. and Farina, B., 2009. Poly (ADP-ribosyl) ation of proteins and germ cell development in hyperthyroid rat testes. *Molecular and cellular biochemistry*, *323*, pp.119-129.
26. Gown, A.M. and Willingham, M.C., 2002. Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase 3. *Journal of Histochemistry & Cytochemistry*, *50*(4), pp.449-454.
27. Griess, P., 1879. Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt „Ueber einige Azoverbindungen" . *Berichte der deutschen chemischen Gesellschaft*, *12*(1), pp.426-428.
28. Hassan, G.S. and Zahran, D.H., 2019. Effects of experimentally induced hyperthyroidism on rat tongue mucosa: Histological and ultrastructural study. *Life Science Journal*, *16*(1), pp.127-134.
29. Ikegami, K., Refetoff, S., Van Cauter, E. and Yoshimura, T., 2019. Interconnection between circadian clocks and thyroid function. *Nature Reviews Endocrinology*, *15*(10), pp.590-600.
30. Jannini, E.A., Crescenzi, A., Rucci, N., Screponi, E., Carosa, E., De Matteis, A., Macchia, E., d'Amati, G. and D'Armiento, M., 2000. Ontogenetic pattern of thyroid hormone receptor expression in the human testis. *The Journal of Clinical Endocrinology & Metabolism*, *85*(9), pp.3453-3457.
31. Jannini, E.A., Ulisse, S. and D'Armiento, M., 1995. Thyroid hormone and male gonadal function. *Endocrine reviews*, *16*(4), pp.443-459.
32. Jayaprakasam, B., Doddaga, S., Wang, R., Holmes, D., Goldfarb, J. and Li, X.M., 2009. Licorice flavonoids inhibit eotaxin-1 secretion by human fetal lung fibroblasts in vitro. *Journal of agricultural and food chemistry*, *57*(3), pp.820-825.
33. Jayaprakasam, B., Doddaga, S., Wang, R., Holmes, D., Goldfarb, J. and Li, X.M., 2009. Licorice flavonoids inhibit eotaxin-1 secretion by human fetal lung fibroblasts in vitro. *Journal of agricultural and food chemistry*, *57*(3), pp.820-825.
34. Kalyanaraman, B., Darley-Usmar, V., Davies, K.J., Dennery, P.A., Forman, H.J., Grisham, M.B., Mann, G.E., Moore, K., Roberts II, L.J. and Ischiropoulos, H., 2012. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free radical biology and medicine*, *52*(1), pp.1-6.
35. Karimani, A., Heidarpour, M. and Moghaddam Jafari, A., 2019. Protective effects of glycyrrhizin on sub-chronic diazinon-induced biochemical, hematological alterations and oxidative stress indices in male Wistar rats. *Drug and chemical toxicology*, *42*(3), pp.300-308.
36. Ko, E.Y., Sabanegh Jr, E.S. and Agarwal, A., 2014. Male infertility testing: reactive oxygen species and antioxidant capacity. *Fertility and sterility*, *102*(6), pp.1518-1527.
37. Kopeina, G.S., Prokhorova, E.A., Lavrik, I.N. and Zhivotovsky, B., 2018. Alterations in the nucleocytoplasmic transport in apoptosis: Caspases lead the way. *Cell Proliferation*, *51*(5), p.e12467.
38. Krajewska-Kulak, E., & Sengupta, P. 2013. Thyroid function in male infertility. *Frontiers in endocrinology*, *4*, 174.
39. Kumar, A., Shekhar, S., & Dhole, B. 2014. Thyroid and male reproduction. *Indian Journal of Endocrinology and Metabolism*, *18*(1), 23.
40. La Vignera, S., & Vita, R. 2018. Thyroid dysfunction and semen quality. *International journal of immunopathology and pharmacology*, *32*, 2058738418775241.
41. Li, X. L., Zhou, A. G., Zhang, L., & Chen, W. J. 2011. Antioxidant status and immune activity of glycyrrhizin in allergic rhinitis mice. *International journal of molecular sciences*, *12*(2), 905-916.
42. Liu, Q., Si, T., Xu, X., Liang, F., Wang, L., & Pan, S. 2015. Electromagnetic radiation at 900 MHz induces sperm apoptosis through bcl-2, bax and caspase-3 signaling pathways in rats. *Reproductive Health*, *12*(1), 1-9.
43. Malekinejad, H., Mirzakhani, N., Razi, M., Cheraghi, H., Alizadeh, A., & Dardmeh, F. 2011. Protective effects of melatonin and Glycyrrhiza glabra extract on ochratoxin A—induced damages on testes in mature rats. *Human & experimental toxicology*, *30*(2), 110-123.

44. Maran, R. R. M. 2003. Thyroid hormones: their role in testicular steroidogenesis. *Archives of andrology*, *49*(5), 375-388.
45. Mennella, M. R. F., Roma, G., & Farina, B. 2003. Active poly (ADPribose) metabolism in DNAase-and salt-resistant rat testis chromatin with high transcriptional activity/competence. *Journal of cellular biochemistry*, *89*(4), 688-697.
46. Mircescu, G. 2008. Oxidative Stress of Chronic Kidney Disease. *Acta Endocrinologica*, *4*(4), 1841-0987.
47. Napoli, C., Paolisso, G., Casamassimi, A., Al-Omran, M., Barbieri, M., Sommese, L., ... & Ignarro, L. J. 2013. Effects of nitric oxide on cell proliferation: novel insights. *Journal of the American College of Cardiology*, *62*(2), 89-95.
48. Narayana, K., D Souza, U. J., & Rao, K. S. 2002. Effect of ribavirin on epididymal sperm count in rat. *Indian journal of physiology and pharmacology*, *46*(1), 97-101.
49. Noh, S., Go, A., Kim, D. B., Park, M., Jeon, H. W., & Kim, B. 2020. Role of antioxidant natural products in management of infertility: a review of their medicinal potential. *Antioxidants*, *9*(10), 957.
50. Orazizadeh, M., Khorsandi, L., Mansouri, E., & Fakhredini, F. 2020. The effect of glycyrrhizin acid on Bax and Bcl2 expression in hepatotoxicity induced by Titanium dioxide nanoparticles in rats. *Gastroenterology and Hepatology from Bed to Bench*, *13*(2), 168.
51. Placer, Z.A., Cushman, L.L. and Johnson, B.C., 1996. Estimation of lipid peroxidation, malindialdehyde in biochemical system. *Anal. Biochem*, *16*, pp.359-367.
52. Rago, R., & Salacone, P. 2008. Hypothalamus-hypophysis-testicle axis. *Andrological Sciences*, *15*(1), 13-17.
53. Rai, G., & Mahobiya, P. 2022. Eventual study of ultraviolet B radiation and their peril on thyroid gland of female Wistar rat. *International Journal of Radiation Research*, *20*(2), 507-510.
54. Rai, S. P., & Vijayalaxmi, K. K. 2001. Tamoxifen citrate induced sperm shape abnormalities in the in vivo mouse. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *492*(1-2), 1-6.
55. Ramadan, H. M., Taha, N. A., & Ahmed, H. H. 2021. Melatonin enhances antioxidant defenses but could not ameliorate the reproductive disorders in induced hyperthyroidism model in male rats. *Environmental Science and Pollution Research*, *28*, 4790-4804.
56. Rao, J. N., Liang, J. Y., Chakraborti, P., & Feng, P. 2003. Effect of thyroid hormone on the development and gene expression of hormone receptors in rat testes in vivo. *Journal of endocrinological investigation*, *26*, 435-443.
57. Rijntjes, E., Wientjes, A. T., Swarts, H. J., de Rooij, D. G., & Teerds, K. J. 2008. Dietary-induced hyperthyroidism marginally affects neonatal testicular development. *Journal of andrology*, *29*(6), 643-653.
58. Sachidhanandam, M., Singh, S.N., Salhan, A.K. and Ray, U.S., 2010. Evaluation of plasma hormone concentrations using Enzyme-Immunoassay/Enzyme-linked Immunosorbent assay in healthy Indian men: Effect of ethnicity. *Indian Journal of Clinical Biochemistry*, *25*, pp.153-157.
59. Sahoo, D. K. 2012. Alterations of testicular selenium-dependent and independent glutathione peroxidase activities during experimentally L-thyroxine induced hyperthyroidism and n-propyl thiouracil induced hypothyroidism in adult rats. *Research & Reviews in BioSciences*, *6*(3).
60. Sahoo, D. K., Roy, A., Bhanja, S., & Chainy, G. B. N. 2005. Experimental hyperthyroidism-induced oxidative stress and impairment of antioxidant defense system in rat testis. *Indian journal of Experimental Biology*, *43*, 1058-1067.
61. Sakr, S. A., & Shalaby, S. Y. 2014. Carbendazim-induced testicular damage and oxidative stress in albino rats: ameliorative effect of licorice aqueous extract. *Toxicology and Industrial Health*, *30*(3), 259-267.
62. Sedlak, J. and Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical biochemistry*, *25*, pp.192-205.
63. Shakyawal, S. and Mahobiya, P. 2023. Repeated UVB Radiations Induced Thyroid Alteration and Oxidative Stress in Male *Swiss albino* Mice. *American Journal of Biomedical Science & Research*, *28*(4).
64. Shakyawal, S., Namdev, N., Ahmad, Z., & Mahobiya, P. 2023. Effects of Ultraviolet B Radiation on the Function of the Testicles, Expression of Caspase-3 and NOS-2, and the Protective Role of Naringin in Mice. *Reproductive Sciences*, *31*(2), 452-468.
65. Sharpe, R. M. 2010. Environmental/lifestyle effects on spermatogenesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1546), 1697-1712.
66. Singh, R., J Hamada, A., & Agarwal, A. 2011. Thyroid hormones in male reproduction and fertility. *The Open Reproductive Science Journal*, *3*(1).
67. Sinha, A.K., 1972. Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), pp.389-394.

68. Spaliviero, J. A., Jimenez, M., Allan, C. M., & Handelsman, D. J. 2004. Luteinizing hormone receptor-mediated effects on initiation of spermatogenesis in gonadotropin-deficient (hpg) mice are replicated by testosterone. *Biology of reproduction*, *70*(1), 32-38.
69. Stefanini, M., Martino, C.D. and Zamboni, L., 1967. Fixation of ejaculated spermatozoa for electron microscopy. *Nature*, *216*(5111), pp.173-174.
70. Venditti, P., & Meo, S. D. 2006. Thyroid hormone-induced oxidative stress. *Cellular and Molecular Life Sciences CMLS*, *63*, 414-434.
71. Wagner, M. S., Wajner, S. M., & Maia, A. L. 2008. The role of thyroid hormone in testicular development and function. *The Journal of endocrinology*, *199*(3), 351.
72. Wajner, S. M., Wagner, M. S., & Maia, A. L. 2009. Clinical implications of altered thyroid status in male testicular function. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *53*, 976-982.
73. Wang, C., Jin, Y., & Jin, Y. 2016. Promoting effect of licorice extract on spermatogonial proliferation and spermatocytes differentiation of neonatal mice in vitro. In Vitro *Cellular & Developmental Biology-Animal*, *52*, 149-155.
74. Xiao, Y., Xu, J., Mao, C., Jin, M., Wu, Q., Zou, J., ... & Zhang, Y. 2010. 18β-glycyrrhetinic acid ameliorates acute Propionibacterium acnes-induced liver injury through inhibition of macrophage inflammatory protein-1α. *Journal of Biological Chemistry*, *285*(2), 1128-1137.
75. Yakymenko, I., Tsybulin, O., Sidorik, E., Henshel, D., Kyrylenko, O., & Kyrylenko, S. 2016. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. *Electromagnetic biology and medicine*, *35*(2), 186-202.
76. Yogianti, F., Kunisada, M., Nakano, E., Ono, R., Sakumi, K., Oka, S., ... & Nishigori, C. 2014. Inhibitory effects of dietary Spirulina platensis on UVB-induced skin inflammatory responses and carcinogenesis. *Journal of Investigative Dermatology*, *134*(10), 2610-2619.