

2022

< Previous Issue | Next Issue >

☰ GO TO SECTION

” Export Citation(s)

ISSUE INFORMATION |  **Free Access**

Issue Information

First Published: 01 April 2022

PDF | **Request permissions**

REVIEW ARTICLE

Physiological role of reactive oxygen species in testis and epididymal spermatozoa

Lizbeth Juárez-Rojas, Fahiel Casillas, Alma López, Miguel Betancourt, Mohammad Mehdi Ommati, Socorro Retana-Márquez

First Published: 16 January 2022

Abstract | **Full text** | **PDF** | **References** | **Request permissions**

Advances in the role of heat shock protein 90 in prostate cancer

Zheng Fu, Benzhong Jia

First Published: 24 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

DNMT enzymes differentially alter global DNA methylation in a stage-dependent manner during spermatogenesis

Fatma Uysal, Gozde Sukur, Ozgur Cinar

First Published: 08 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Use of *Tribulus terrestris* and *Lepidium meyenii* extract in rats: reproductive, biochemical and body parameters

David Richard Miranda, Bruna Resende Chaves, Lara Vilela Soares, Isaac Filipe Moreira Konig, Juliana Tensol Pinto, José Rafael Miranda, Márcio Gilberto Zangeronimo

First Published: 28 December 2021

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Role of shear wave elastography (SWE) in erectile dysfunction patients for evaluation of daily tadalafil treatment outcome

Khaled M. Abdelwahab, Mohamed Salah Eldery, Esam Desoky, Islam M. El-Babouly, Kareem Taha, Sameh Saber, Mohammed M. Seleem

First Published: 12 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

Association between the international index of erectile function-15 and female sexual function index in Chinese infertile couples

Qiushuang Wang, Hao Geng, Caiyun Lu, Zonglan Jin, Chuan Xu, Dongdong Tang

First Published: 04 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Ameliorative effects of a curcumin vitamin E nanocomposite coated with olive oil against cadmium chloride-induced testicular damage

Entsar R. Abd-Allah, Heba Ali Abd El-Rahman

First Published: 30 December 2021

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Inhibition of human sperm function by an antibody against apolipoprotein A1: A protein located in human spermatozoa

Xiuping Chi, Daijun Xiang, Yingjiao Sha, Shuang Liang, Chengbin Wang

First Published: 05 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Learning curve of ThuLEP (Thulium laser enucleation of the prostate): Single-centre experience on initial consecutive 60 patients

Tahsin Batuhan Aydogan, Murat Binbay

First Published: 04 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Oral thymoquinone modulates cyclophosphamide-induced testicular toxicity in adolescent Wistar rats

Misturah Y. Adana, Aminu Imam, Ahmed A. Bello, Olawale E. Sunmonu, Ezekiel P. Alege, Oluwafemi G. Onigbolabi, Moyosore Salihu Ajao

ORIGINAL ARTICLE

Sinapic acid ameliorates cisplatin-induced disruptions in testicular steroidogenesis and spermatogenesis by modulating androgen receptor, proliferating cell nuclear antigen and apoptosis in male rats

Murat Demir, Fikret Altındağ

First Published: 08 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE | [Open Access](#)

German version of the Hypogonadism Impact of Symptoms Questionnaire: Psychometric properties and association with testosterone levels

Martina Kathrin Schmidt, Julia Rohayem, Michael Zitzmann, Heather Gelhorn, Gudrun Schneider

First Published: 21 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Clinical research analysis based on prostate cancer screening diagnosis

Jiahao Shan, Xinyu Geng, Ziyang Liu, Youlu Lu, Raorao Zhou, Zhengyuan Zhang, Haoran Xu, Xiaojie Zhou, Wenzhuo Ma, Hengyu Zhu, Hongbin Shi

First Published: 11 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Prospective study on blood pressure, lipid metabolism and erythrocytosis during and after androgen abuse

Diederik L. Smit, Aldo Grefhorst, Madelon M. Buijs, Olivier de Hon, Martin den Heijer, Willem de Ronde

First Published: 11 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Mehmet Yilmaz, Yusuf Sahin, Ibrahim Hacıbey, Unsal Ozkuvanci, Serhat Suzan, Ahmet Yaser Muslumanoglu

First Published: 18 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Minocycline can reduce testicular apoptosis related to varicocele in male rats

Majid Shokoohi, Arash Khaki, Ayda Roudi Rasht Abadi, Linda MohammadZadeh Boukani, Sepideh Hassanpour Khodaie, Hossein Kalarestaghi, Amir Afshin Khaki, Maryam Moghimian, Hamid Reza Niazkar, Hamed Shoorei

First Published: 09 March 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

Evaluating the utility of the A.F. Genital System test for pathogen diagnosis in acute male urethritis

Mehmet Sarier, Nevgun Sepin, Mestan Emek, Aysegül Tezcan Germen, Mustafa Burak Hoscan, Elcin Konuk, Hasan Turgut

First Published: 22 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

ORIGINAL ARTICLE

The therapeutic effects of distinct exercise types on metabolic syndrome-induced reproductive system impairment in male rats: Potential contribution of mitochondria-related genes

Muhammed Emre Karaman, Ahmet Tektemur

First Published: 04 February 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

Fear about adverse effect on fertility is a major cause of COVID-19 vaccine hesitancy in the United States

Parris Diaz, John Zizzo, Navin C. Balaji, Rohit Reddy, Kajal Khodamoradi, Jesse Ory, Ranjith Ramasamy

First Published: 30 December 2021

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

CASE REPORT

Primary spindle cell sarcoma of the seminal vesicle: A case report and literature review

Yufeng Song, Hao Ning, Zhigang Yao, Haihu Wu, Jinglong Shao, Maowen Fan, Jiaju Lu

First Published: 05 January 2022

[Abstract](#) | [Full text](#) | [PDF](#) | [References](#) | [Request permissions](#)

Sign up for email alerts

Enter your email to receive alerts when new articles and issues are published.

Email address

[Continue](#)

Tools

 [Submit an article](#)

[Back](#)

Researchers Using AI

A study by WILEY



Journal Metrics

Related Titles



in Research Jobs

Please [contact us](#) to see your job listed here

[More jobs ►](#)



ABOUT WILEY ONLINE LIBRARY

[Privacy Policy](#)

[Terms of Use](#)

[About Cookies](#)

[Manage Cookies](#)

[Accessibility](#)

[Wiley Research DE&I Statement and Publishing Policies](#)

[Developing World Access](#)

HELP & SUPPORT

[Contact Us](#)

[Training and Support](#)

[DMCA & Reporting Piracy](#)

OPPORTUNITIES

[Subscription Agents](#)

[← Back](#)


The Wiley Network
Wiley Press Room

Copyright © 1999-2025 John Wiley & Sons, Inc or related companies. All rights reserved, including rights for text and data mining and training of artificial intelligence technologies or similar technologies.

ORIGINAL ARTICLE

Oral thymoquinone modulates cyclophosphamide-induced testicular toxicity in adolescent Wistar rats

Misturah Y. Adana  | Aminu Imam | Ahmed A. Bello | Olawale E. Sunmonu | Ezekiel P. Alege | Oluwafemi G. Onigbolabi | Moyosore Salihu Ajao

Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

Correspondence

Misturah Y. Adana, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

Emails: misturahadana@gmail.com, adana.my@unilorin.edung

Abstract

Cyclophosphamide (CYP) is an effective anti-cancer drug that is widely accepted, but it is not devoid of unintended toxic effects. Gonadal toxicity is reported as one of the side effects of its long-time use. This study examined the effects of thymoquinone (TQ) on the biological integrities of the testes after cyclophosphamide exposure. Thirty adolescent male Wistar rats (100–110 g) were divided into six groups ($n = 5$), receiving normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5), and 20 mg/kg of CYP and 10 mg/kg of TQ (CTQ10) respectively. On the 22nd day, blood, semen and testicular samples were collected for the assay of serum reproductive hormones (follicle-stimulating (FSH) and luteinizing (LH) hormones), semen analysis and testicular histology and proliferating cell nuclear antigen (PCNA) expression. The results revealed that CYP exposure affected functional and structural integrities of the testes, by depleting sperm count and motility, testosterone, LH, spermatogenic and mature sperm cell population, Leydig cells and PCNA immunoreactive proliferating cells. TQ interventions were able to reverse all cytotoxic CYP impacts, but with differential activities on the hormonal concentrations, specifically LH and FSH. Cumulatively, thymoquinone may be a potent agent against cyclophosphamide effects on the physiological, regeneration and histological integrities of the testes, as observed in this study.

KEYWORDS

cyclophosphamide, gonadotoxicity, proliferating cell nuclear antigen, semen analysis, thymoquinone

1 | INTRODUCTION

Infertility is the inability to achieve conception in the reproductive age with no known or perceived pathology (Tabong & Adongo, 2013), with growing concerns in the facility lagging developing nations (Bala et al., 2021; Bhattacharya & Johnson, 2010; Mascarenhas et al., 2012). More disturbing is that, most reproductive challenges have no known identifiable causes, marred by unclear pathologies. Exogenous agents, including pharmacological drugs, essential

environmental chemicals and some occupational exposures have been linked to contributing to the burden of infertility (Dutta et al., 2021; Kumar, 2018; Marić et al., 2021).

Cyclophosphamide (CYP) is one of the most successful chemotherapeutic agents available and is still extensively used in a wide range of diseases, alone and in combination with other therapies. These conditions include lupus, rheumatoid arthritis, neoplasms, nephrotic syndrome and conditioning of regimens for blood and marrow transplantation (Emadi et al., 2009; Sarici et al., 2021). The early

clinical trials of cyclophosphamide for the treatment of neoplasms were carried out in 1958, and in 1959 (Brock & Wilmanns, 1958), and subsequently became one of the first sets of cancer chemotherapeutic agents approved by the food and drug authority (Muggia et al., 1980). Although CYP is widely recognized as an effective anticancer drug to date (Ahlmann & Hempel, 2016), it is not without undesirable toxic effects, earlier reported (Fraiser et al., 1991; Hill et al., 1972) and recently recorded in biological systems in the body (Bhat et al., 2018; Brown et al., 2021; Huang et al., 2021).

Its teratogenic effects on the male systems were reported decades back and have continued to get attention due to its continued use (Briseño-Bugarín et al., 2021; Saxena & Kumar, 2020; Trasler et al., 1986), with some researchers reporting depleted sperm population and an absence of spermatogenic cycles in the testicular tissue and damages to spermatogenic cells and impaired fertility in adult male patients treated with cyclophosphamide (Nayak et al., 2016). All of these will not reduce its use, due to the documented effectiveness, it is therefore imperative to find means of reversing or protecting against the gonadotoxic effect of this very useful drug.

Plants are essential parts of cultural, traditional and pharmaceutical medical adventures for centuries, and their use in bioactive forms is regaining popularity in the management of varying diseases in the last decades. One of the plants with increasing use in recent times is *Nigella sativa*, popularly known as black seed (Ahmad et al., 2021). Researchers have identified that a lot of the pharmacological activities of *Nigella sativa* are credited to the most active nutrient—thymoquinone a bioactive flavonoid (Tavakkoli et al., 2017). The reported pharmacological activities of the plant include but are not limited to antioxidant (Iqbal et al., 2021), anti-inflammatory (Bordoni et al., 2019), immunomodulatory (Khazdair et al., 2021; Shaterzadeh-Yazdi et al., 2018), neuroprotective (Fanoudi et al., 2019; Landucci et al., 2021) and fertility-enhancing (Razak et al., 2017; Shikhbahaei et al., 2018; Wani et al., 2022) capacities, among others. Hence, this experiment aims to examine the probable protective potential of thymoquinone against cyclophosphamide-induced testicular toxicity using animal models.

2 | MATERIALS AND METHODS

2.1 | Ethics approval

This research work was approved by the University of Ilorin ethical review committee (UERC) (UERC/ASN/2019/856), upon the recommendation of the College of Health Sciences ethical review committee, in compliance with the Institutional Animal Care and Use Committee (IACUC).

2.2 | Chemicals and drugs

Thymoquinone was purchased from Sigma-Aldrich, while the cyclophosphamide was purchased at Aromokeye Pharmaceutical Company, Ilorin, Kwara State, Nigeria.

2.3 | Animal care

Thirty adolescent male Wistar rats weighing 110–120 g were collected from Ilorin's University of Ilorin Biological Garden. They were kept in the animal house facility of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, and fed a normal laboratory meal and water ad libitum. The rats were put into a 12-h light/dark cycle at room temperature. They were cared for and used following the standard laboratory animal care and use guide.

2.4 | Experimental design

Thirty 6-week-old, male Wistar rats were randomly placed into six groups of five rats each. They were handled for two (2) weeks before the experiment began, for familiarization with the new conditions. The six groups include, Group NS, which received normal saline; CYP, received cyclophosphamide 20 mg/kg; TQ5, received thymoquinone 5 mg/kg; TQ10, received thymoquinone 10 mg/kg; CTQ5, received cyclophosphamide 20 mg/kg and thymoquinone 5 mg/kg; and CTQ10, received cyclophosphamide 20 mg/kg and thymoquinone 10 mg/kg. All administrations were via the oral route. Cyclophosphamide and thymoquinone were mixed with normal saline to form solutions for easy dosing and administration. The rats were weighed weekly for dosage adjustments, and the experiment lasted 28 days.

2.5 | Experimental termination and sample collection

The rats were euthanized a day after the treatments under anaesthesia by ketamine. Blood was collected, centrifuged and semen separated for hormonal assay, while testes were excised, weighed, fixed in Bouin's fluid and subsequently processed for histological and immunohistochemical analysis. Also, the cauda epididymis of each rat was excised and gently minced in 2 ml of normal saline for semen analysis. The resulting suspension was used for the determination of sperm motility according to the methods of Tjioe and Oentoeng (1968). A drop of the suspension on the glass slide was viewed under the light microscope. The percentage of motile spermatozoa was derived by subtracting the number of still spermatozoa from the total sperm count. Spermatozoa exhibiting any motion after the dilution were considered motile.

Sperm concentration was determined by immobilizing cells in 3% saline. This was then thoroughly mixed and assessed using a Makler Counting Chamber (Sef-Medical Instruments Ltd Haifa) according to the method of Eliasson (1975). A sperm suspension of 10 μ l was placed onto the Makler Chamber stage and counted for the number of sperm under a light microscope (Olympus).

TABLE 1 Body weight changes in experimental animals

Group	Initial body weight	Final body weight (FBW)	Weight changes	Testicular weight (TW)	Relative testicular weight (RTW) = TW/FBW × 100
NS	103.2 ± 12.23	121.6 ± 20.74	18.40 ± 5.24 [*]	1.39 ± 0.12	1.10 ± 0.12
CYP	112.4 ± 15.86	100.8 ± 21.45	-12.80 ± 6.03	1.22 ± 0.10	1.20 ± 0.07
TQ5	118.4 ± 15.28	145.8 ± 18.19	27.40 ± 6.78 [*]	1.28 ± 0.22	0.90 ± 0.12
TQ10	142.8 ± 14.29	175.4 ± 10.43	32.60 ± 13.96 [*]	1.38 ± 0.08	0.80 ± 0.12
CTQ5	176.0 ± 4.20	199.6 ± 13.08	23.60 ± 9.71 [*]	1.20 ± 0.05	0.62 ± 0.06
CTQ10	147.4 ± 6.28	138.0 ± 6.08	-9.60 ± 3.87	1.43 ± 0.16	0.98 ± 0.08

*Significance when compared to CYP group ($p < 0.05$).

2.6 | Reproductive hormones assay

The reproductive hormones follicle-stimulating hormone (FSH), testosterone and luteinizing hormone (LH) levels were assayed using a commercially available kit (IBL) and the manufacturer's instructions. The kit employs a competition-based solid-phase enzyme-linked immunosorbent assay (ELISA). The samples were plated in duplicate, and the concentrations of testosterone (catalogue no. CSB-E05100r), FSH (catalogue no. CSB-E12654r) and LH (catalogue no. CSB-E06869r) were determined using a standard curve with 5 standard values. Using a microplate reader (Tecan Infinite M1000 Pro), the optical density of the samples and testosterone, FSH and LH standards were quantified at 450 nm.

2.7 | Histological and morphometric studies

For histopathological examination, fixed testicular tissues were dissected, processed through graded ethanol and embedded in paraffin. Microtome sections at 5 µm were obtained and stained with haematoxylin and eosin using a microtome (micron HM 315 microtome). The slices were examined and images were captured using an Olympus light microscope (Olympus BX51) and a camera (Olympus E330, Olympus Optical Co. Ltd.). Adopting a systematic random sampling, a total of eleven seminiferous tubules were carefully chosen for each rat, and Image J software was used to estimate measure the lumen and total seminiferous tubule diameters (Adana et al., 2018; Howard & Reed, 2004; Osinubi et al., 2005).

2.8 | Proliferative nuclear cell antigen studies

The PCNA study was done using immunohistochemical studies. In each group, five blocks per testis for the PCNA study were immunostained. The carefully chosen paraffin blocks for immunohistochemical studies were stained with monoclonal antibody PCNA (LabVision, avidin-biotin-peroxidase method). Deparaffinized sections were rehydrated with graded alcohols after being deparaffinized in xylol. They were then immersed in 0.01 M citrate buffer (pH 6) and microwaved (Gibson's oven) on high for 20 min before coming for at least 20 min at room temperature. Endogenous peroxidase activity was inhibited by a 10-min incubation in phosphate buffer

saline with 3% hydrogen peroxide (PBS, 10 mM Na₂PO₄, 140 mM NaCl, pH 7.2). The sections were rinsed three times for 2 min each before beginning the immunostaining process (Foley et al., 1991).

Histostainplus kit (Zymed) immunohistochemical staining was conducted using the streptavidin-biotin method, which contains 10% nonimmune serum and secondary antibody (biotinylated) and Streptavidin-peroxidase. Sections were rinsed multiple times with PBS before being treated with blocking serum for 10 min to inhibit unspecific binding. After removing the excess blocking serum, the histological sections were coated with primary antibody PCNA in dilution (1:50) for one hour at room temperature. For 10 min, the secondary antibody was utilized as a second layer. Following that, the sections were washed for 10 min with PBS and streptavidin-peroxidase complex. Following that, the sections were rinsed with PBS, and the peroxidase signal was generated in phosphate buffer saline containing 0.05% diaminobenzidine (DAB) and 0.01% hydrogen peroxide (H₂O₂). Before drying and mounting with per mount, the sections were faintly counter-stained in haematoxylin. Negative control slides were created by replacing the primary antibody with PBS in identical areas. In the same run, slides with normal positive reactivity tissues of the antibody were also employed. Nuclear staining was observed in PCNA expression instances that were brown (Foley et al., 1991).

2.9 | Statistical analysis

SPSS version 18 was used to analyse the data. The obtained results were provided as mean SD, and all statistical comparisons were performed using one-way analysis of variance (ANOVA). The significance test was also used to see if there were any significant changes between the treatments (the level of significance was set at $p \leq 0.05$ and $p \leq 0.01$).

3 | RESULTS

3.1 | CYP toxicity induced emaciation and lethargy in rats

Weekly weighing of rats revealed significant physical changes in the weight of the animals. The rats in group CYP experienced a significant loss in body weight when compared to groups NS, TQ5 and

TQ10. When compared to group CYP, the rats in groups CTQ5 and CTQ10 displayed lesser weight loss. There was no significant difference in the relative testicular weights (see Table 1). Rats treated with CYP were observed to be sluggish and relatively inactive when compared to rats in NS, TQ5 and TQ10 groups.

3.2 | Thymoquinone ingestion prevented semen composite depletion in cyclophosphamide-induced testicular toxicity

Repeated CYP exposure caused significant ($p < 0.05$) reductions in semen reproductive viability, by depleting the sperm population and affecting their motility when compared to the control and all TQ treated rats (Figures 1 and 2). The above activities were, however, observed to be prevented by TQ interventions (in CTQ5 and CTQ10), by markedly ($p < 0.05$) preserving sperm population and their motility when compared to the cyclophosphamide only exposed rats (Figures 1 and 2).

3.3 | Differential hormonal responses following cyclophosphamide and thymoquinone ingestions

The testosterone level of the rats exposed to CYP were significantly low when compared to the control ($p < 0.05$). Surprisingly, TQ at 5 mg/kg alone and in the CYP exposed rats preserved and stimulated increased testosterone levels, while the 10 mg/kg dose was not only seen to not prevent the depletion caused by CYP, it further caused the reduction (Figure 3). Thus, only the lower dose of TQ rescues testosterone in this study. In like manner to the effects of the 10 mg/kg TQ on testosterone concentration, the two

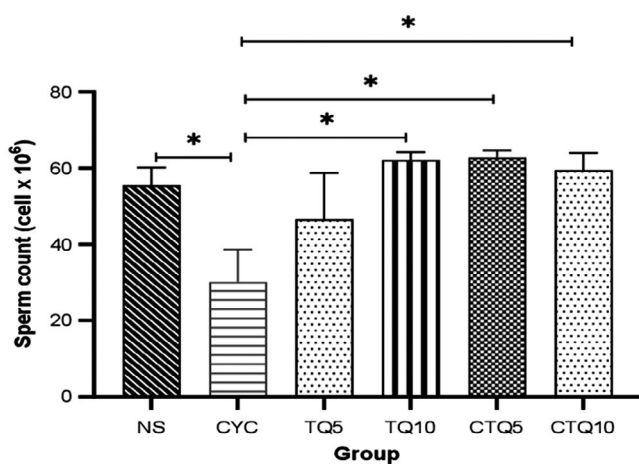


FIGURE 1 Sperm counts in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5), and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD. Asterisk (*) represents a statistically significant difference ($p < 0.05$)

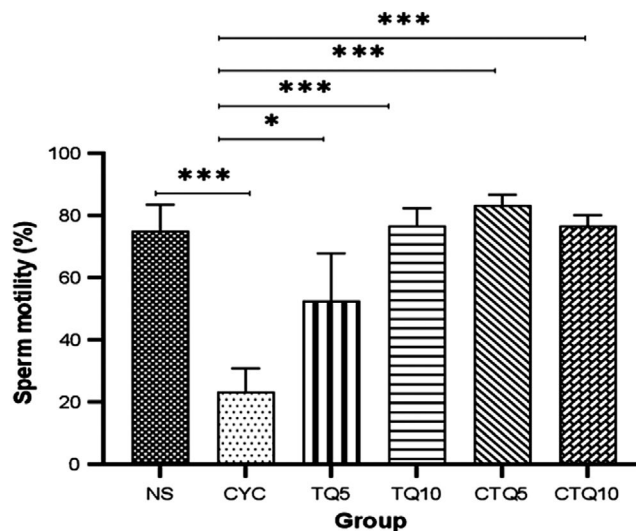


FIGURE 2 Sperm motility in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5), and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD, while single and triple asterisks (*) and (***) represents statistically significant difference ($p < 0.05$) between the groups

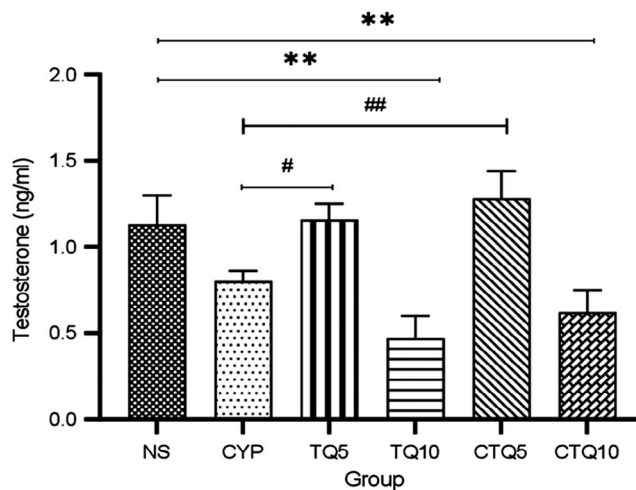


FIGURE 3 Serum testosterone in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5), and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD, while single asterisk (*) represents statistically significant difference at $p < 0.05$, double (**) represents significance at $p < 0.01$ between the control and other groups, while single and double # signifies difference at $p < 0.05$ and $p < 0.01$ between the groups and CYP only group

doses (5 and 10 mg/kg) of TQ used in this study caused significant ($p < 0.05$) reduction in serum LH concentrations individually and/or in the CYP-exposed rats (Figure 4). Lastly, repeated oral CYP resulted in significantly higher serum FSH concentrations in the exposed rats when compared to the control (Figure 5). Correlative to TQ activities on testosterone concentrations, the 5 and 10 mg/kg of

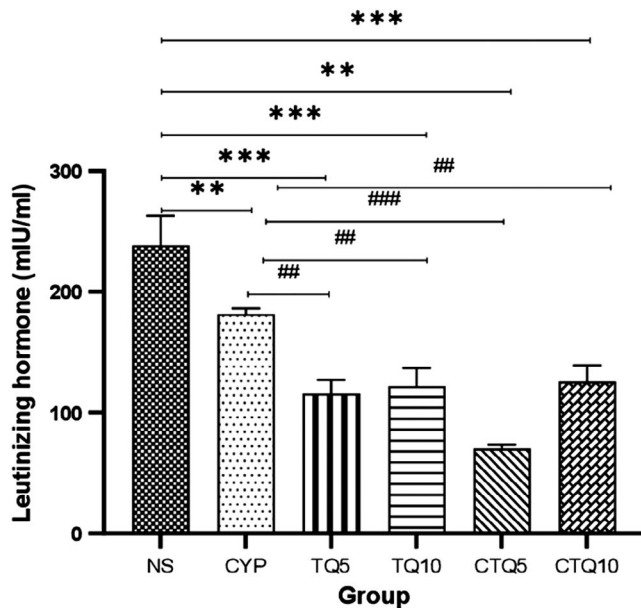


FIGURE 4 Serum luteinizing hormone in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5), and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD, double asterisks (**) represents statistically significant difference at $p < 0.01$, triple asterisks (***) represents significance at $p < 0.001$ between the control and other groups, while double and triple # signifies difference at $p < 0.01$ and $p < 0.001$ between the groups and CYP only group

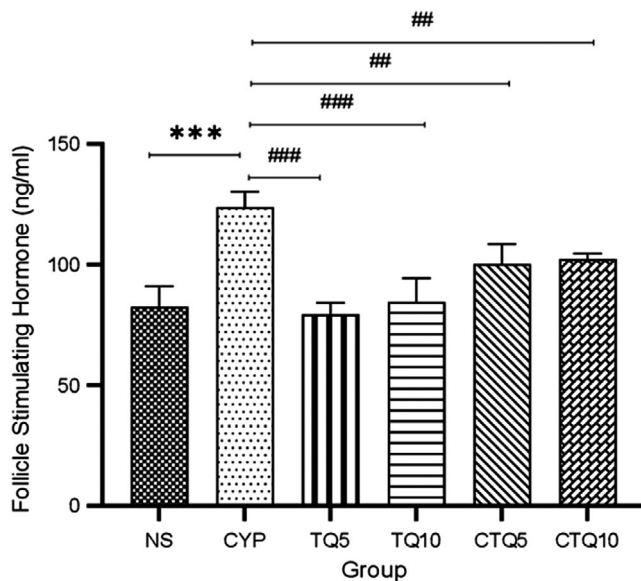


FIGURE 5 Serum follicle-stimulating hormone in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5) and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD, triple asterisks (***) represents significance at $p < 0.001$ between the control and other groups, while double and triple # signifies difference at $p < 0.01$ and $p < 0.001$ between the groups and CYP only group

TQ sufficiently reduced FSH concentrations in the TQ-only treated rats and in the CYP-exposed rats (Figure 5).

3.4 | Thymoquinone preserved testicular histoarchitectural integrities and morphometry in CYP toxicity

Microscopic examination of the testicular histology as shown in Figure 6 revealed that rats treated with normal saline, thymoquinone alone and as interventions, after CYP had normal testicular cytoarchitecture with spermatogenic cells at different stages of development. However, in the CYP-exposed rats are observable histopathological alterations within the seminiferous tubules, marked by the reduction in identifiable spermatogenic cells and matured sperm cells in the lumina of the seminiferous tubules and the Leydig cells in the interstitium (Figure 6). Also, the thickness of the germinal epithelium in the rats exposed to CYP reduced markedly ($p < 0.05$) when compared to the control, while TQ interventions dose-dependently preserved the germinal epithelial thickness in the treated rats (Figure 7).

3.5 | Thymoquinone prevents the loss of proliferative cells in CYP-induced testicular toxicity

The number of cells with proliferative nuclear antigen (PCNA) were markedly ($p < 0.05$) lost in the testes of the rats repeatedly exposed to CYP when compared to the saline, 5 mg kg⁻¹ and 10 mg kg⁻¹ TQ treated rats. The interventions with low dose (5 mg kg⁻¹) and high dose (10 mg kg⁻¹) TQ effectively preserved the population of the PCNA immunoreactive proliferative cells when compared to the CYP alone and were seen to stimulate increased proliferation when compared to the control rats (Figures 8 and 9).

4 | DISCUSSION

The maturation of sperm cells begins with the differentiation of spermatogonia in the seminiferous tubules, which after a series of events transform into the mature spermatozoa. For viable spermatozoa with the reproductive capability to be produced, spermatogenesis must go unhindered and undisturbed. An insult to the body or specifically directed at the reproductive organs, such as the testes, could distort spermatogenesis and impair reproductive functions in males, due to the poor quality and characteristics of sperm cells (Azhar et al., 2021; Badkoobeh et al., 2013; Omotoso et al., 2017; Tvrdá et al., 2021).

Cyclophosphamide is a cytotoxic agent that has been extensively used for the treatment of various diseases, most specifically in cancer (Comenzo et al., 2020; Noori et al., 2020; Perini et al., 2007; Sugita, 2019). It has, however, been shown to have gonadotoxic properties (Fukushima et al., 2005; Ghobadi et al., 2017). It has been shown

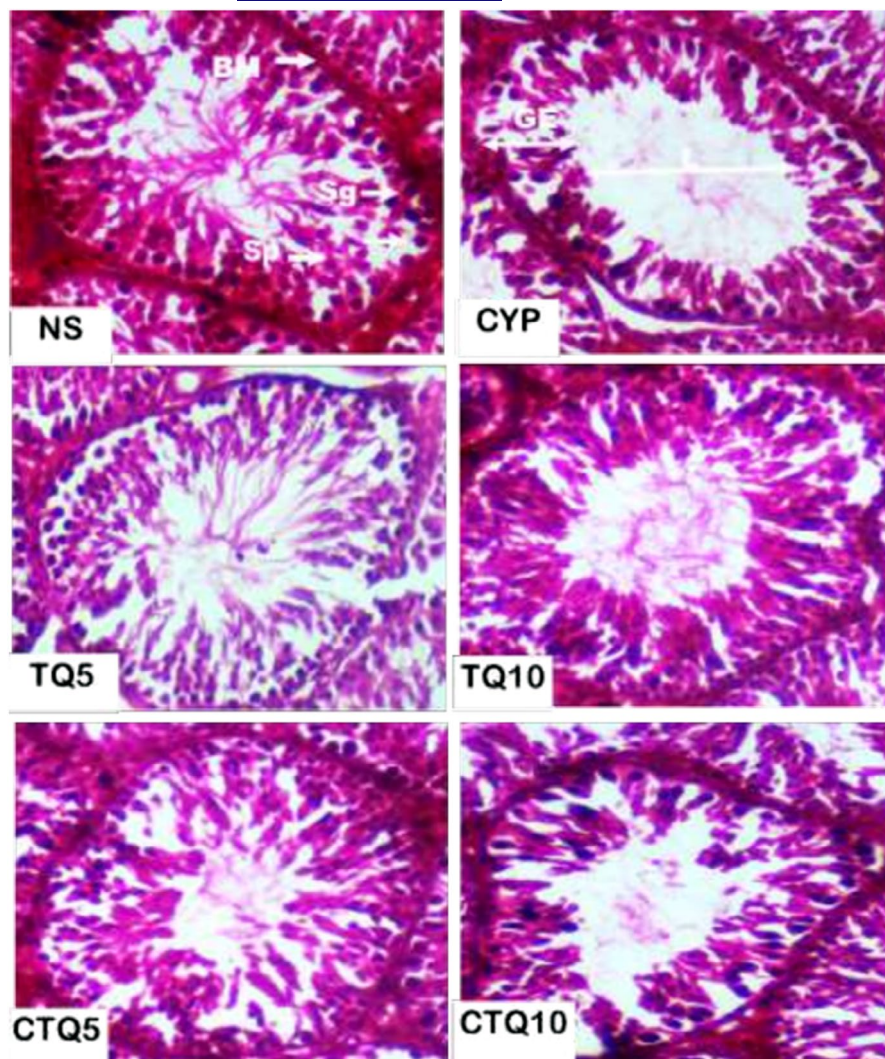


FIGURE 6 Representative photomicrographs of testis in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5) and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. BM, basement membrane; GE, germinal epithelium; I, interstitium; L, lumen; Sg, spermatogonia. H&E stain, $\times 100$

that the testicular dysfunction observed is a known long-term consequence of cytotoxic chemotherapy used in the treatment of many cancers (Drobnis & Nangia, 2017; Salian et al., 2020). This study observed histopathological alterations within the seminiferous tubules. These were marked by the reduction in identifiable spermatozoa in the lumina of the seminiferous tubules and the Leydig cells in the interstitium. These findings have been established in both human (Łazowski et al., 1982; Meirow & Nugent, 2001; Tian En et al., 2020) and animal studies (Elangovan et al., 2006; Gadallah & Hanan, 2020; Torabi et al., 2017). Co-administration with cyclophosphamide and thymoquinone showed improved testicular histomorphology—there were increases in the thickness of the germinal epithelium and the population of mature cells in the lumen of the seminiferous tubules. The seminiferous tubules displayed increases in the thickness of the germinal epithelium and also a reduction in the diameter of the central lumen.

The testicular parenchyma was made of many distorted, shrunken seminiferous tubules with a widened lumen in the seminiferous tubules of rats. There was a marked reduction in the thickness of the germinal epithelium and wide empty lumina. This reduction in the number of stratified epithelial layers has been attributed to

inhibition of B-spermatogonia mitosis which denotes elongation of the G1 phase of cell cycle growth cycle (Anan et al., 2017; McGeady et al., 2017). Furthermore, it has been shown that this thinning of the epithelium leads to a defect in sperm production (Anan et al., 2017). Clinically, these sperm defects are manifested by oligospermia or azoospermia which give rise to infertility. Earlier observations showed that administration of CYP once a week for 5 weeks caused oligospermia, azoospermia, testicular damage and germ cell toxicity in mice (Badkoobeh et al., 2013). According to other studies, increased morphological imperfection and production of abnormal spermatozoa may also be a result of the direct toxicity of CYP, because cellular DNA is a primary mechanism of CYP anti-cancer and toxic abilities; thus, DNA damage may be responsible for the increased level of abnormal spermatozoa forms (Mehraban et al., 2021). Another mechanism for CYP toxicity on sperm morphology may also be the peroxidation of polyunsaturated fatty acids in plasma membranes of spermatozoa by free radicals and low antioxidant capacity. Adenosine triphosphate (ATP) is an energy source for sperm motility and the reduced sperm motility following CYP treatment may be the result of a disruption to ATP supply (Ahmed et al., 2015; Badkoobeh et al., 2013).

The testosterone and luteinizing hormone levels in animals treated with CYP were shown to be highly impacted by CYP. This was reflected in the semen quality. Results from this study show a

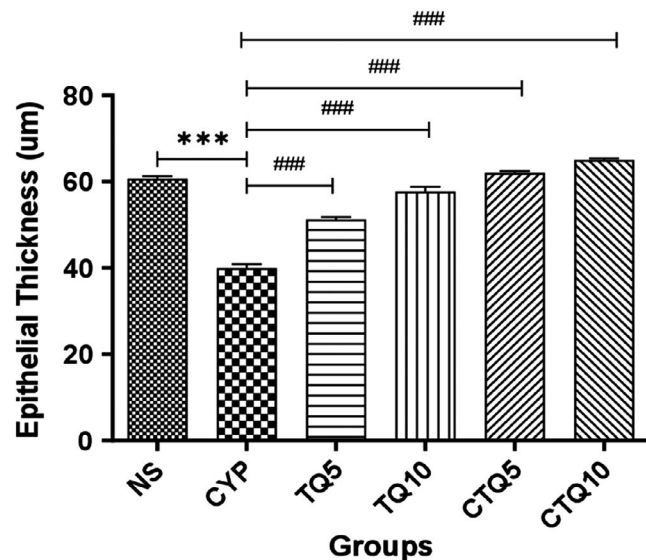


FIGURE 7 Germinal epithelial thickness in rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5) and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD, triple asterisks (***) represents significance at $p < 0.001$ between the control and other groups, while double and triple # signifies difference at $p < 0.001$ between the groups and CYP only group

significant decrease in sperm count and progressively motile spermatozoa with the use of CYP. The drop observed in the sperm count and actively motile spermatozoa were noteworthy; however, treatment with thymoquinone was able to achieve some degree of restoration. Furthermore, many reports have revealed that TQ plays a role in increasing Leydig cell numbers and testosterone levels which eventually improve spermatogenesis (Negi et al., 2018). Thymoquinone similarly exerts enhanced antioxidant activities that can ameliorate the spermatogenesis impairment caused by testicular injury through oxidative stress. Previous studies demonstrated the protective roles of TQ on impaired spermatogenesis resulting from chronic toluene exposure (Negi et al., 2018) and suggested that TQ could ameliorate the deleterious effects of cadmium chloride by activating the testicular endocrine and antioxidant system (Sayed et al., 2014). Its antioxidant defence against the generation of reactive oxygen species (ROS) suggested its protective activity in the testicular tissue (Negi et al., 2018).

The role of proliferating cell nuclear antigen (PCNA) in the testes is well-established. It plays a vital role in nucleic acid metabolism as a key player in cell replication and repair. In this study, the expression of PCNA in the CYP-administered groups was diminished. This is indicative of reduced cellular replication due to damage to the actively dividing portion of the germinal epithelium lined along the basement membrane of the seminiferous tubules-spermatogonic cells and the primary spermatocytes. Damage to the mitotic germinal epithelium is also indicative of DNA insults. While some researchers believe the positive expression of PCNA is indicative of a good response to spermatogenesis dysfunction (Zhao et al., 2018), others suggest that it is an indication of well-regulated spermatogenesis (Fadlalla et al.,

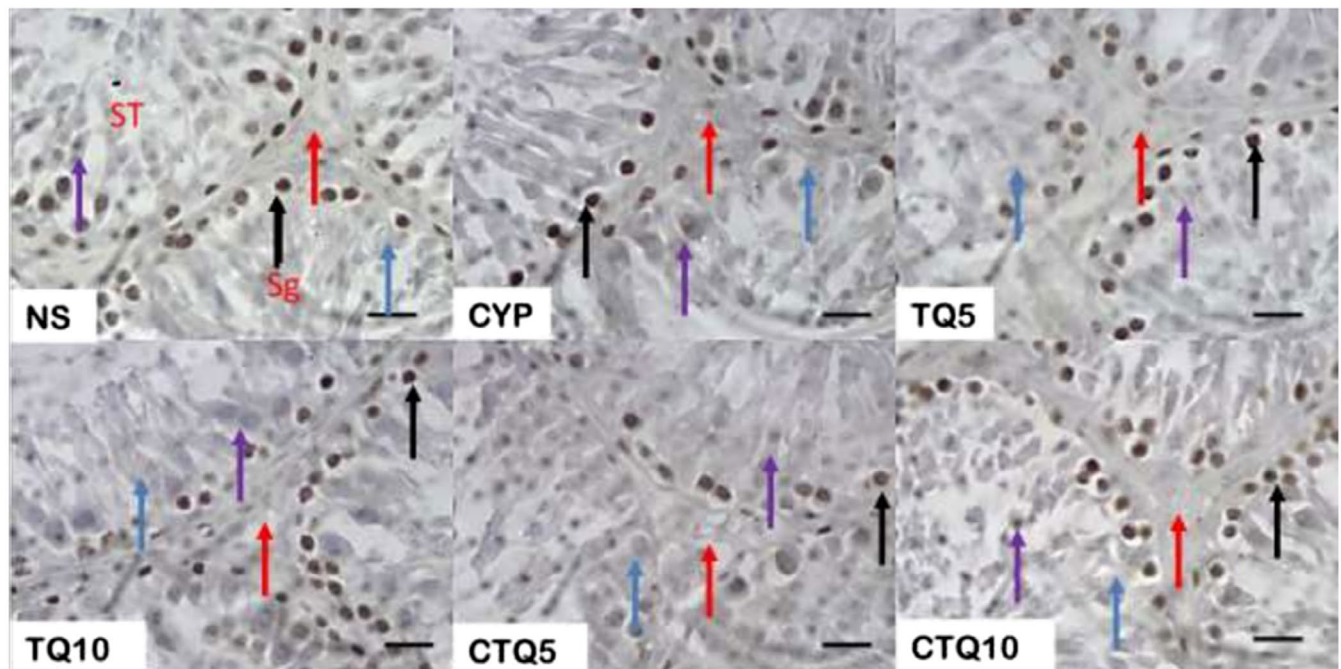


FIGURE 8 Representative photomicrographs of the distributions of proliferative nuclear antigen (PCNA) immunoreactive cells in the testis of rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5) and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. ST, seminiferous tubule, Early germ cells along the basement membrane (spermatogonia - Sg) are deeply stained (black arrows), whereas several spermatocytes show less intense (purple arrows) staining. Sertoli cells (blue arrows) and interstitial cells of Leydig (Red arrows) stains negative for PCNA. $\times 100$, 50 μ m

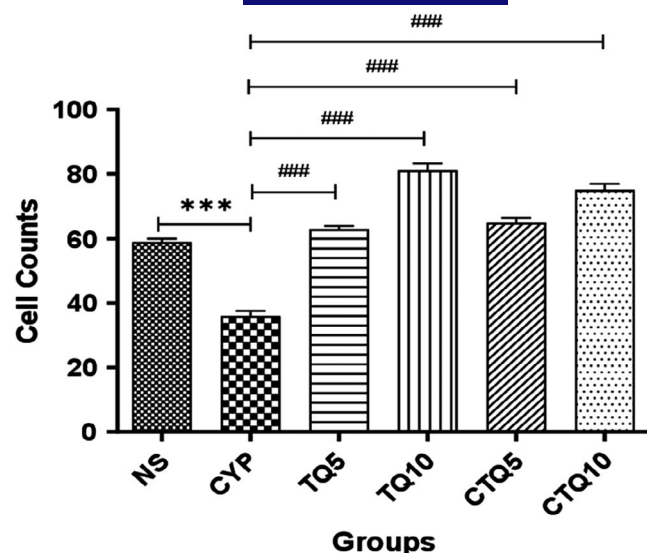


FIGURE 9 proliferative nuclear antigen (PCNA) immunoreactive cells count in the testis of rats exposed to normal saline (NS), 20 mg/kg of CYP (CYP), 5 mg/kg of TQ (TQ5), 10 mg/kg of TQ (TQ10), 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ5) and 20 mg/kg of CYP and 5 mg/kg of TQ (CTQ10) respectively. Bars indicate mean $\pm$ SD, triple asterisks (***) represents significance at $p < 0.001$ between the control and other groups, while double and triple # signifies difference at $p < 0.001$ between the groups and CYP only group

2017; Miura et al., 2002). Co-administration of high dose TQ (10 mg/kg) and CYP displayed expressions similar to control. This aligns with findings suggesting that positive expression of PCNA is a pointer to testicular well-being.

Thymoquinone has been proven to show a wide range of favourable biological activities, the most prominent being antioxidant (Entok et al., 2014) and anti-inflammatory (Ahmad et al., 2021; Ashraf et al., 2011). Co-administration with CYP was shown to limit these structural and biochemical changes in the gonads.

5 | CONCLUSION

Thymoquinone, the main bioactive ingredient of *Nigella sativa* oil, has displayed the potential ability to protect against cyclophosphamide-induced gonadal toxicity. It may therefore be a promising adjuvant supplement in modulating the gonadotoxicity associated with cyclophosphamide use. However, more work needs to be done to give a better understanding of the role of thymoquinone.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Misturah Y. Adana  <https://orcid.org/0000-0001-8538-7838>

REFERENCES

- Adana, M. Y., Akang, E. N., Naidu, E. C. S., Aniekan, P. I., Kouame, K., Offor, U., Ogedengbe, O. O., & Azu, O. O. (2018). Testicular micro-anatomical and hormonal alterations following use of antiretroviral therapy in Sprague Dawley rats: Role of Naringenin. *Andrologia*, 50(9), e13137.
- Ahlmann, M., & Hempel, G. (2016). The effect of cyclophosphamide on the immune system: Implications for clinical cancer therapy. *Cancer Chemotherapy and Pharmacology*, 78(4), 661–671. <https://doi.org/10.1007/s00280-016-3152-1>
- Ahmad, M. F., Ahmad, F. A., Ashraf, S. A., Saad, H. H., Wahab, S., Khan, M. I., Ali, M., Mohan, S., Hakeem, K. R., & Athar, M. T. (2021). An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. *Journal of Herbal Medicine*, 25, 100404.
- Ahmed, L. A., El-Maraghy, S. A., & Rizk, S. M. (2015). Role of the KATP channel in the protective effect of nicorandil on cyclophosphamide-induced lung and testicular toxicity in rats. *Scientific Reports*, 5(1), 1–11. <https://doi.org/10.1038/srep14043>
- Anan, H. H., Wahba, N. S., Abdallah, M. A., & Mohamed, D. A. (2017). Histological and immunohistochemical study of cyclophosphamide effect on adult rat testis. *International Journal of Scientific Reports*, 3(2), 39–48. <https://doi.org/10.18203/issn.2454-2156.IntJSciRep20170356>
- Ashraf, S. S., Rao, M. V., Kaneez, F. S., Qadri, S., Al-Marzouqi, A. H., Chandranath, I. S., & Adem, A. (2011). *Nigella sativa* extract is a potent antioxidant for petrochemical-induced oxidative stress. *Journal of Chromatographic Science*, 49(4), 321–326.
- Azhar, M., Altaf, S., Uddin, I., Cheng, J., Wu, L., Tong, X., Qin, W., & Bao, J. (2021). Towards post-meiotic sperm production: Genetic insight into human infertility from mouse models. *International Journal of Biological Sciences*, 17(10), 2487–2503. <https://doi.org/10.7150/ijbs.60384>
- Badkoobeh, P., Parivar, K., Kalantar, S. M., Hosseini, S. D., & Salabat, A. (2013). Effect of nano-zinc oxide on doxorubicin-induced oxidative stress and sperm disorders in adult male Wistar rats. *Iranian Journal of Reproductive Medicine*, 11(5), 355.
- Bala, R., Singh, V., Rajender, S., & Singh, K. (2021). Environment, lifestyle, and female infertility. *Reproductive Sciences*, 28(3), 617–638. <https://doi.org/10.1007/s43032-020-00279-3>
- Bhat, N., Kalthur, S. G., Padmashali, S., & Monappa, V. (2018). Toxic effects of different doses of cyclophosphamide on the liver and kidney tissue in Swiss albino mice: A histopathological study. *Ethiopian Journal of Health Sciences*, 28(6).
- Bhattacharya, S., Johnson, N., Tijani, H. A., Hart, R., Pandey, S., & Gibreel, A. F. (2010). Female infertility. *Clinical Evidence*.
- Bordoni, L., Fedeli, D., Nasuti, C., Maggi, F., Papa, F., Wabitsch, M., De Caterina, R., & Gabbianelli, R. (2019). Antioxidant and anti-inflammatory properties of *Nigella sativa* oil in human preadipocytes. *Antioxidants*, 8(2), 51. <https://doi.org/10.3390/antiox8020051>
- Briseño-Bugarín, J., Hernández-Ochoa, I., Araujo-Padilla, X., Mojica-Villegas, M. A., Montaña-González, R. I., Gutiérrez-Salmeán, G., & Chamorro-Cevallos, G. (2021). Phycobiliproteins ameliorate gonadal toxicity in male mice treated with cyclophosphamide. *Nutrients*, 13(8), 2616. <https://doi.org/10.3390/nu13082616>
- Brock, N., & Wilmanns, H. (1958). Effect of a cyclic nitrogen mustard-phosphamide on experimentally induced tumors in rats; chemotherapeutic effect and pharmacological properties of B 518 ASTA. *Deutsche Medizinische Wochenschrift*, 83(12), 453–458.

- Brown, T., Sykes, D., & Allen, A. R. (2021). Implications of breast cancer chemotherapy-induced inflammation on the gut, liver, and central nervous system. *Biomedicines*, 9(2), 189.
- Comenzo, R. L., Kastritis, E., Palladini, G., Minnema, M. C., Wechalekar, A. D., Jaccard, A., Santhorawala, V., Lee, H. C., Gibbs, S. D., & Mollee, P. (2020). *Reduction in absolute involved free light chain and difference between involved and uninvolved free light chain is associated with prolonged major organ deterioration progression-free survival in patients with newly diagnosed AL amyloidosis receiving bortezomib, cyclophosphamide, and dexamethasone with or without daratumumab: Results from Andromeda*. American Society of Hematology.
- Drobnis, E. Z., & Nangia, A. K. (2017). Immunosuppressants and male reproduction. *Impacts of Medications on Male Fertility*, 179–210.
- Dutta, S., Gorain, B., Choudhury, H., Roychoudhury, S., & Sengupta, P. (2021). Environmental and occupational exposure of metals and female reproductive health. *Environmental Science and Pollution Research*, 1–26.
- Elangovan, N., Chiou, T.-J., Tzeng, W.-F., & Chu, S.-T. (2006). Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. *Toxicology*, 222(1–2), 60–70.
- Eliasson, R. (1975). Analysis of semen. *Progress in Infertility*, 39.
- Emadi, A., Jones, R. J., & Brodsky, R. A. (2009). Cyclophosphamide and cancer: Golden anniversary. *Nature Reviews Clinical Oncology*, 6(11), 638–647.
- Entok, E., Ustuner, M. C., Ozbayer, C., Tekin, N., Akyuz, F., Yangi, B., Kurt, H., Degirmenci, I., & Gunes, H. V. (2014). Anti-inflammatory and anti-oxidative effects of *Nigella sativa* L.: 18 FDG-PET imaging of inflammation. *Molecular Biology Reports*, 41(5), 2827–2834.
- Fadlalla, M. B., Wei, Q., Fedail, J. S., Mehfooz, A., Mao, D., & Shi, F. (2017). Effects of hyper- and hypothyroidism on the development and proliferation of testicular cells in prepubertal rats. *Animal Science Journal*, 88(12), 1943–1954.
- Fanoudi, S., Alavi, M. S., Hosseini, M., & Sadeghnia, H. R. (2019). *Nigella sativa* and thymoquinone attenuate oxidative stress and cognitive impairment following cerebral hypoperfusion in rats. *Metabolic Brain Disease*, 34(4), 1001–1010.
- Foley, J. F., Dietrich, D. R., Swenberg, J. A., & Maronpot, R. R. (1991). Detection and evaluation of proliferating cell nuclear antigen (PCNA) in rat tissue by an improved immunohistochemical procedure. *Journal of Histochemistry*, 14(4), 237–241.
- Fraiser, L. H., Kanekal, S., & Kehr, J. P. (1991). Cyclophosphamide toxicity. *Drugs*, 42(5), 781–795.
- Fukushima, T., Kato, M., Adachi, T., Hamada, Y., Horimoto, M., Komiyama, M., Mori, C., & Horii, I. (2005). Effects of sulfasalazine on sperm acrosome reaction and gene expression in the male reproductive organs of rats. *Toxicological Sciences*, 85(1), 675–682.
- Gadallah, M., & Hanan, N. (2020). The protective role of atorvastatin against the damage induced by cyclophosphamide on the kidney and testis of adult male albino rat: Histological, biochemical and histomorphometric study. *The Medical Journal of Cairo University*, 88(September), 1867–1882.
- Ghobadi, E., Moloudizargari, M., Asghari, M. H., & Abdollahi, M. (2017). The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. *Expert Opinion on Drug Metabolism & Toxicology*, 13(5), 525–536.
- Hill, D. L., Laster, W. R., & Struck, R. F. (1972). Enzymatic metabolism of cyclophosphamide and nicotine and production of a toxic cyclophosphamide metabolite. *Cancer Research*, 32(4), 658–665.
- Howard, V., & Reed, M. (2004). *Unbiased stereology: Three-dimensional measurement in microscopy*. Garland Science.
- Huang, S., Yang, J., Fu, F., Wang, C., Guo, X., He, B., Xiao, D., Cai, H., & Liu, M. (2021). Clinical and genetic risk factors for the prediction of hepatotoxicity induced by docetaxel, epirubicin, and cyclophosphamide regimen in breast cancer patients. *Pharmacogenomics*, 22(2), 87–98.
- Iqbal, M. S., Iqbal, Z., Hashem, A., Al-Arjani, A.-B.-F., Abd-Allah, E. F., Jafri, A., Ansari, S. A., & Ansari, M. I. (2021). *Nigella sativa* callus treated with sodium azide exhibits augmented antioxidant activity and DNA damage inhibition. *Scientific Reports*, 11(1), 1–14.
- Khazdair, M. R., Ghafari, S., & Sadeghi, M. (2021). Possible therapeutic effects of *Nigella sativa* and its thymoquinone on COVID-19. *Pharmaceutical Biology*, 59(1), 696–703.
- Kumar, S. (2018). Occupational and environmental exposure to lead and reproductive health impairment: An overview. *Indian Journal of Occupational and Environmental Medicine*, 22(3), 128.
- Landucci, E., Mazzantini, C., Buonvicino, D., Pellegrini-Giampietro, D. E., & Bergonzi, M. C. (2021). Neuroprotective effects of thymoquinone by the modulation of ER stress and apoptotic pathway in in vitro model of excitotoxicity. *Molecules*, 26(6), 1592.
- Łazowski, Z., Janczewski, Z., & Polowiec, Z. (1982). The effect of alkylating agents on the reproductive and hormonal testicular function in patients with rheumatoid arthritis. *Scandinavian Journal of Rheumatology*, 11(1), 49–54.
- Marić, T., Fučić, A., & Aghayanian, A. (2021). Environmental and occupational exposures associated with male infertility. *Archives of Industrial Hygiene and Toxicology*, 72(3), 101–113.
- Mascarenhas, M. N., Flaxman, S. R., Boerma, T., Vanderpoel, S., & Stevens, G. A. (2012). National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. *PLoS Med*, 9(12), e1001356.
- McGeady, T. A., Quinn, P. J., Fitzpatrick, E., Ryan, M., Kilroy, D., & Lonergan, P. (2017). *Veterinary embryology*. John Wiley & Sons.
- Mehraban, Z., Novin, M. G., Golmohammadi, M. G., & Nazarian, H. (2021). Effect of *Ceratonia siliqua* L. extract on DNA fragmentation of sperm in adult male mice treated with cyclophosphamide. *Reproductive Sciences*, 28(4), 974–981.
- Meirow, D., & Nugent, D. (2001). The effects of radiotherapy and chemotherapy on female reproduction. *Human Reproduction Update*, 7(6), 535–543.
- Miura, C., Miura, T., & Yamashita, M. (2002). PCNA protein expression during spermatogenesis of the Japanese eel (*Anguilla japonica*). *Zoological Science*, 19(1), 87–91.
- Muggia, F., Henney, J., & DeVita, V. (1980). General progress in clinical chemotherapy of advanced disease. *Cancer Chemo- and Immunopharmacology*, 1–7.
- Nayak, G., Vadinkar, A., Nair, S., Kalthur, S. G., D'Souza, A. S., Shetty, P., Mutalik, S., Shetty, M., Kalthur, G., & Adiga, S. (2016). Sperm abnormalities induced by pre-pubertal exposure to cyclophosphamide are effectively mitigated by *Moringa oleifera* leaf extract. *Andrologia*, 48(2), 125–136.
- Negi, P., Rathore, C., Sharma, G., Singh, B., & Katore, O. P. (2018). Thymoquinone a potential therapeutic molecule from the plant *Nigella sativa*: Role of colloidal carriers in its effective delivery. *Recent Patents on Drug Delivery & Formulation*, 12(1), 3–22.
- Noori, S., Tavirani, M. R., Deravi, N., Rabbani, M. I. M., & Zarghi, A. (2020). Naringenin enhances the anti-cancer effect of cyclophosphamide against MDA-MB-231 breast cancer cells via targeting the STAT3 signaling pathway. *Iranian Journal of Pharmaceutical Research*, 19(3), 122.
- Omotoso, G. O., Hambolu, O. Z., & Alabi, A. S. (2017). Cigarette smoke alters testicular and epididymal histology in adult wistar rats.
- Osinubi, A. A., Noronha, C. C., & Okanlawon, A. O. (2005). Morphometric and stereological assessment of the effects of long-term administration of quinine on the morphology of rat testis. *West African Journal of Medicine*, 24(3), 200–205.
- Perini, P., Calabrese, M., Rinaldi, L., & Gallo, P. (2007). The safety profile of cyclophosphamide in multiple sclerosis therapy. *Expert Opinion on Drug Safety*, 6(2), 183–190.
- Razak, R. N. H. A., Isa, M. L. M., Muhammad, H., & Ramli, R. (2017). Animal studies on male fertility-enhancing properties of plants in Malaysia:

- A review of the past 16 years. *International Journal of Allied Health Sciences*, 1(2), 44–55.
- Salian, S. R., Uppangala, S., Cheredath, A., D'souza, F., Kalthur, G., Nayak, V. C., Anderson, R. A., & Adiga, S. K. (2020). Early prepubertal cyclophosphamide exposure in mice results in long-term loss of ovarian reserve, and impaired embryonic development and blastocyst quality. *PLoS One*, 15(6), e0235140.
- Sarici, A., Erkurt, M. A., Kuku, I., Gok, S., Bahceciloglu, O. F., Bicim, S., Berber, I., Kaya, E., & Ozgul, M. (2021). Selection of the mobilization regimen in lymphoma patients: A retrospective cohort study. *Transfusion and Apheresis Science*, 60(5), 103251.
- Saxena, A. K., & Kumar, A. (2020). Effect of cyclophosphamide on testis—A histological study. In M. Dieter (Ed.), *Fish analysis for drug and chemicals mediated cellular toxicity* (pp. 43–58). Springer.
- Sayed, M. M., Hassanein, K. M., & Senosy, W. (2014). Protective effects of thymoquinone and L-cysteine on cadmium-induced reproductive toxicity in rats. *Toxicology Reports*, 1, 612–620.
- Shaterzadeh-Yazdi, H., Noorbakhsh, M.-F., Hayati, F., Samarghandian, S., & Farkhondeh, T. (2018). Immunomodulatory and anti-inflammatory effects of thymoquinone. *Cardiovascular & Haematological Disorders-Drug Targets*, 18(1), 52–60.
- Shikhbahaei, F., Ghanbari, A., & Keshtmand, Z. (2018). Therapeutic effect of thymoquinone against methotrexate-induced damage on sperm parameters in mice Efecto terapéutico de la tiroquinona contra el daño inducido por metotrexato en los parámetros espermáticos en ratones. *International Journal of Morphology*, 36(2), 519–522.
- Sugita, J. (2019). HLA-haploidentical stem cell transplantation using post-transplant cyclophosphamide. *International Journal of Hematology*, 110(1), 30–38.
- Tabong, P.-T.-N., & Adongo, P. B. (2013). Understanding the social meaning of infertility and childbearing: A qualitative study of the perception of childbearing and childlessness in Northern Ghana. *PLoS One*, 8(1), e54429.
- Tavakkoli, A., Mahdian, V., Razavi, B. M., & Hosseinzadeh, H. (2017). Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. *Journal of Pharmacopuncture*, 20(3), 179.
- Tian En, L., Brougham, M. F., Wallace, W. H. B., & Mitchell, R. T. (2020). Impacts of platinum-based chemotherapy on subsequent testicular function and fertility in boys with cancer. *Human Reproduction Update*, 26(6), 874–885.
- Tjioe, D., & Oentoeng, S. (1968). The viscosity of human semen and the percentage of motile spermatozoa. *Fertility and Sterility*, 19(4), 562–565.
- Torabi, F., Shafaroudi, M. M., & Rezaei, N. (2017). Combined protective effect of zinc oxide nanoparticles and melatonin on cyclophosphamide-induced toxicity in testicular histology and sperm parameters in adult Wistar rats. *International Journal of Reproductive BioMedicine*, 15(7), 403.
- Trasler, J. M., Hales, B. F., & Robaire, B. (1986). Chronic low dose cyclophosphamide treatment of adult male rats: Effect on fertility, pregnancy outcome and progeny. *Biology of Reproduction*, 34(2), 275–283.
- Tvrđá, E., Benko, F., Slanina, T., & du Plessis, S. S. (2021). The role of selected natural biomolecules in sperm production and functionality. *Molecules*, 26(17), 5196.
- Wani, J. A., Tsagkaris, C., Majid, S., Ganie, M. A., Akhter, R., Ahmad, S. B., Ovais, S., Khan, M. S., & Wani, H. A. (2022). In K. Andleeb, U. R. Muneeb (Eds.), *Therapeutic effects of Nigella sativa on hormonal dysfunctions*. In *Black Seeds (Nigella sativa)* (pp. 217–238). Elsevier.
- Zhao, W. P., Wang, H. W., Liu, J., Tan, P. P., Luo, X. I., Zhu, S. Q., Chen, X. I., & Zhou, B. H. (2018). Positive PCNA and Ki-67 expression in the testis correlates with spermatogenesis dysfunction in fluoride-treated rats. *Biological Trace Element Research*, 186(2), 489–497.

How to cite this article: Adana, M. Y., Imam, A., Bello, A. A., Sunmonu, O. E., Alege, E. P., Onigbolabi, O. G., & Salihu Ajao, M. (2022). Oral thymoquinone modulates cyclophosphamide-induced testicular toxicity in adolescent Wistar rats. *Andrologia*, 00, e14368. <https://doi.org/10.1111/and.14368>