Trop J Nat Prod Res, May 2023; 7(5):3223-3229 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Ameliorative Potential of Thymoquinone on Male Reproductive Functions in Lead Acetate-Induced Reproductive Toxicity in Male Wistar Rats

Kabiru I. Adedokun[a]*, Olayemi O. Oladokun[a], Tunde F. Abraham[b], Opeyemi S. Osuntokun[c], Tope G. Atere[d], Sakinat T. Baiyewu[e]

[a]*Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University Osogbo, Nigeria.*
[b]*Department of Physiology, Prince Abubakar Audu University, Anyigba, Kogi State.*
[c]*Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Federal University of Oye-Ekiti, Ekiti State, Nigeria.*
[d]*Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University Osogbo, Nigeria.*
[e]*Department of Health, Well-being, and Social Care, Global Banking School/Oxford Brookes University Partnership, Leeds, England.*

ARTICLE INFO

Article history:
Received 14 February 2023
Revised 14 May 2023
Accepted 24 May 2023
Published online 01 July 2023

ABSTRACT

Lead reproductive toxicity has been reported in men. *Nigella sativa* seed and its active component, thymoquinone have been used in traditional pharmacopeia as natural remedy to promote reproductive health. This study investigated the ameliorative potential of Thymoquinone (TQ) on male reproductive functions in lead acetate (PbA)-induced reproductive toxicity in Wistar rats. Thirty male Wistar rats weighing 100–120 g were randomly divided into six groups (n=5); Control (distilled water, 0.2 ml/day), PbA, PbA+5 mg/kg TQ, PbA+3.75 mg/kg TQ, PbA+2.5 mg/kg and 5 mg/kg TQ. TQ was given orally at varying doses daily for 8 weeks while PbA (15 mg/Kg) was administered intraperitoneally once per week for 8 weeks. Hypothalamic-pituitary-testicular hormones, sperm analysis, and histological studies of the testis and epididymis were conducted and data were analyzed. A significant decrease ($p<0.05$) in GnRH, FSH, LH, and testosterone in PbA, PbA+3.75 mg/kg TQ, and PbA+2.5 mg/kg TQ when compared with the control group. However, GnRH, FSH, LH, and testosterone significantly increased ($p<0.05$) in PbA+5 mg/kg TQ and PbA+3.75 mg/kg TQ when compared with PbA treated group. Sperm count, motility, viability, and morphology showed significant decrease in PbA treated group when compared with the control, while these were significantly ameliorated following TQ administration. Cytoarchitectural distortions observed in the testis and epididymis in PbA-treated groups were equally improved with TQ administration. Lead acetate induces reproductive toxicity in male Wistar rats via a reduction in hypothalamic-pituitary-testicular hormones, sperm parameters as well as distortion of both testicular and epididymal cytoarchitectures. However, Thymoquinone ameliorated these anomalies.

***Keywords*:** Lead acetate, thymoquinone, hypothalamic-pituitary-gonadal hormones, sperm parameters

Introduction

Heavy metal toxicity is a major threat to public health throughout the world. Considerably, Lead affects almost all the major organ systems of the body.[1] Several kinds of research have associated lead to a wide range of adverse reproductive outcomes. In men, lead can reduce the libido and affect spermatogenesis reducing the quality of sperm.[2] Human exposure to lead through the environment has increased several folds due to its wide range of applications in industries, cosmetics, and medicine. As shown by several studies, lead-induced toxicity is through oxidative stress mechanisms as a result of the production of reactive oxygen species (ROS).[3]

Infertility is a global health issue that affects approximately 10% -15% of couples trying to conceive and male factor infertility accounts for almost half of the cases.[4]

*Corresponding author. E mail: kabiru.adedokun@uniosun.edu.ng
Tel: +2348036559099

Citation: Adedokun KI, Oladokun OO, Abraham TF, Osuntokun OS, Atere TG, Baiyewu ST. Ameliorative Potential of Thymoquinone on Male Reproductive Functions in Lead Acetate-Induced Reproductive Toxicity in Male Wistar Rats. Trop J Nat Prod Res. 2023; 7(5):3223-3229 http://www.doi.org/10.26538/tjnpr/v7i6.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Lead is revealed to have unfavorable results on reproductive function, particularly in males.[5] Therefore, this study is one of the concerted efforts in search of alternative ideal curative substances against the deleterious effects of lead-acetate-induced nephrotoxicity particularly in males.

Among many medicinal plants, *Nigella sativa* (*Ranunculaceae* herbaceous plant), is a medicinally promising herb that has been researched and is well-known for its pharmacological benefits. The seeds of *Nigella sativa* known as black seed or black cumin are being used as medicine globally having anti-oxidant, anti-fertility, anti-inflammatory gastro-protective, anti-tumor, anti-anxiety, and anti-microbial properties.[6,7] Black seed has an active effect on reproductive functions and infertility treatment as it has been demonstrated to cause significant improvements in reproductive parameters such as sperm count, semen qualities, Leydig cells count, follicular development, corpus luteum, and gonadotropic hormones like testosterone and progesterone.[8] Nigella sativa has many different chemical ingredients including thymoquinone (30-48%), flavonoids, anthocyanins, alkaloids, and essential fatty acids, particularly linoleic and oleic acids.[9]

Thymoquinone (TQ), the main antioxidant constituent of *Nigella sativa* seed oil has shown beneficial effects on semen quality and reproductive functions. Several reports have shown that TQ plays a role to increase Leydig cell number and testosterone levels which eventually improved spermatogenesis.[9,10,11] Thymoquinone also exerts marked antioxidant characteristics which could ameliorate the spermatogenesis impairment

caused by testicular injury through oxidative stress.[12] However, the role of TQ against lead acetate-induced nephrotoxicity in male Wistar rats has not been fully elucidated. Therefore, the present study investigated the ameliorative role of TQ on male reproductive functions in lead acetate-induced reproductive toxicity in male Wistar rats.

Materials and Methods

Experimental animals
Thirty male Wistar rats (100-120 grams) were housed in well-ventilated cages in the Animal House Unit of the College of Health Sciences, Osun State University, Osogbo, with constant 12-h light 12-h dark cycle. The animals had free access to standard pelletized rat feed and clean water *ad libitum* and were allowed two weeks of acclimatization. All the procedures in this study followed the College of Health Sciences, Research Ethics Committee, Osun State University, Osogbo, with reference number: uniosunhrec2022B/022

Experimental design
The animals were weighed and randomly shared into six groups of five animals each. Control group (A) and group F were given 0.2 ml/kg of distilled water and 5 mg/kg of Thymoquinone (TQ) respectively. Reproductive toxicity was induced intraperitoneal by administration of Lead acetate-PbA (15 mg/kg) in groups B (PbA only), C (PbA+5 mg/kg TQ), D (PbA+3.75 mg/kg TQ) and E (PbA+2.5 mg/kg TQ). PbA was administered once per week and TQ daily throughout the duration (56 days) of the study. The dosage of PbA was in concordance with the method of Gosh *et al*.,[13] with little modification while that of Thymoquinone follows the method of Tekeoglu *et al*.,[14]

Sacrifice and Sample Collection
The rats were sacrificed on the 56th day by intraperitoneal injection of 120 mg/kg of sodium thiopentone anesthesia.[15] Blood samples for serum hormonal assay were collected from each animal via cardiac puncture into the plain bottle. Reproductive organs were excised, cleared of fatty and connective tissues, and weighed to the nearest milligram. Organs for histological studies were preserved in Bouin's fluid.

Sperm analysi:
Sperm characteristics analysis was conducted on the spermatozoa collected from caudal epididymis using an Olympus research microscope (Olympus, Japan) under the x40 objective lens of the microscope. Progressive motility was assessed immediately and scored to the nearest percentage. A sperm viability study (percentage of live sperm) was done using the eosin/nigrosin staining technique.[16] The live sperm cells were unstained while the non-motile (dead) sperm absorbed the stain. Sperm count and morphology were done using the method established by Seed *et al*.[17]

Hormone assay
Enzyme-linked Immunosorbent Assay (ELISA) kits (Calbiotech, USA) were used to determine the concentration of GnRH, testosterone, LH, and FSH, in the serum samples collected.

Histological studies
The testis and epididymis were fixed in Bouin's fluid before being dehydrated in descending grades of alcohol, cleared in chloroform, and impregnated in paraffin. Then 5-6 µm sections were placed into the grease-free slides, de-paraffinized in xylene, and stained with hematoxylin and eosin. Morphological changes were then observed under a light microscope at x400 magnification. This was carried out according to the previous method of Adedokun *et al*.[18]
Statistical Analysis: Data is expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using a Student t-test and one-way analysis of variance (ANOVA). Differences between the groups with a p-value < 0.05 were considered significant. Data were analyzed with the use of GraphPad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA).

Results and Discussion

Effects of Thymoquinone on Relative Reproductive Organs weight in Lead acetate-Induced Reproductive Toxicity in Male Wistar Rats
There was no significant difference in the relative mean weight of testis and epididymis in groups B to F when compared with the control (Table 1).
Lead poisoning, one of the most prominent poisonings among heavy metals has been implicated in male reproductive dysfunctions by several studies.[19,20,21] However, Thymoquinone, the main constituent of *Nigella sativa* seed oil has been reported to demonstrate significant improvement in semen qualities, reproductive functions, and sexual hormones.[9,10,11] This study examined the ameliorative potentials of Thymoquinone on male reproductive functions in lead acetate-induced reproductive toxicity in male Wistar rats.
In this study, the relative mean testicular and epididymal weight of Wistar rats showed no significant differences across the different treatment groups when compared to the control group. This finding is partly consistent with the study of Wadi *et al.*,[22] who reported no significant differences in testicular weight in lead-induced repro-toxicity; however, reported that the high-dose treatment significantly decreased the epididymal weight, suggesting the tendency of this toxicant to induce underweight or even emaciation in mammals. The weight of the male reproductive organs usually provides a useful reproductive risk assessment in experimental studies.[23]

Effects of Thymoquinone on Sperm Parameters in Lead acetate-Induced Reproductive Toxicity in Male Wistar Rats
There was a significant decrease in percentage sperm motility, viability, morphology, and count in PbA treated group, PbA+5 mg/kg TQ, PbA+3.75 mg/kg TQ, and PbA+2.5 mg/kg TQ compared to the control. However, sperm motility, viability, morphology, and count in PbA+5 mg/kg TQ, PbA+3.75 mg/kg TQ, PbA+2.5 mg/kg TQ and 5 mg/kg TQ groups significantly increased when compared with PbA treated group (Table 2).
Sperm acquire their motile ability during their epididymal transit and in the normal course, all caudal epididymal spermatozoa are motile.[24] PbA-induced repro-toxicity on caudal epididymal sperm motility, viability, count, and morphology as shown in this study demonstrate moderate to severe interference with functional competence and structural integrity of the spermatozoa. These observations may be a result of the decreased antioxidant levels, increase in reactive oxygen species (ROS) generation, and interruption on the hypothalamic-pituitary axis caused by PbA as earlier observed by other researchers.[25,26] This is equally in line with previous reports that lead to significant decreases in sperm indices causing reproductive toxicity.[27,28,29] However, TQ reverses all the adverse effects observed on the sperm indices. *Nigella sativa* oil and its active constituents have been reported by several authors to improve sperm parameters.[12,30,31] Mahdavi *et al*.,[31] reported that the antioxidant properties of Thymoquinone may be responsible for the neutralized free radicals in semen and improved sperm parameters.

Table1: Effect of Thymoquinone on Testicular and Epididymal weight in lead acetate-induced reproductive toxicity in male Wistar Rats

Groups	Relative Testicular weight (%)	Relative Epididymal Weight (%)
A (Control)	0.99 ± 0.16	0.14 ± 0.02
B (PbA)	0.91 ± 0.32	0.18 ± 0.05
C (PbA+5 mg/kg TQ)	0.83 ± 0.04	0.14 ± 0.01
D (PbA+3.75 mg/kg TQ)	0.69 ± 0.16	0.13 ± 0.03
E (PbA+2.5 mg/kg TQ)	0.68 ± 0.07	0.12 ± 0.02
F (5 mg/kg TQ)	0.71 ± 0.0	0.18 ± 0.04

No significant difference

Table 2: Effect of Thymoquinone on sperm parameters in lead acetate-induced reproductive toxicity in male Wistar Rats

Groups	Motility (%)	Viability (%)	Count (x10^6/mL)	Morphology (%)
A (Control)	80.00 ± 2.04	76.25 ± 2.39	213.00 ± 11.81	89.00 ± 1.29
B (PbA)	45.00 ± 2.04^a	50.00 ± 2.04^a	103.40 ± 4.93^a	59.75 ± 4.03^a
C (PbA+5 mg/kg TQ)	68.75 ± 1.25^{ab}	66.25 ± 1.25^{ab}	193.50 ± 4.51^{ab}	76.50 ± 5.12^a
D (PbA+3.75 mg/kg TQ)	61.25 ± 3.14^{ab}	62.50 ± 2.50^{ab}	142.30 ± 4.82^{ab}	71.50 ± 1.85^a
E (PbA+2.5 mg/kg TQ)	60.00 ± 2.04^{ab}	61.25 ± 2.39^{ab}	132.50 ± 6.45^{ab}	61.25 ± 2.09^a
F (5 mg/kg TQ)	80.00 ± 2.04^b	73.35 ± 2.39^b	217.50 ± 13.10^b	85.25 ± 3.25^b

a: significant decrease (P<0.05) when compared with the control group.
b: significant increase (P<0.05) when compared with PbA groups.

Effects of Thymoquinone on hypothalamic-pituitary-testicular Hormones in Lead acetate-Induced Reproductive Toxicity in Male Wistar Rats

There was a significant decrease ($p < 0.05$) in GnRH in PbA, PbA + 3.75 mg/kg TQ, and PbA+ 2.5 mg/kg TQ treated groups when compared with the control group. No significant difference was observed in gonadotropin-releasing GnRH in PbA+ 5mg/kg TQ and 5 mg/kg TQ treated groups when compared with the control. However, there was a significant increase ($p < 0.05$) in GnRH in PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, 5 mg/kg TQ treated groups when compared with PbA treated groups. Conversely, no significant difference in the level of GnRH in the PbA+2.5 mg/kg TQ-treated group when compared with PbA-treated groups (Figure 1)

LH showed a significant decrease ($p < 0.05$) in PbA, PbA + 5 mg/kg TQ, PbA+3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ when compared with control. There was a significant increase ($P < 0.05$) in LH level in PbA + 5 mg/kg TQ, PbA+3.75 mg/kg TQ, and 5 mg/kg TQ when compared with PbA treated groups. While no significant difference in LH was observed in PbA + 2.5 mg/kg TQ when compared with PbA treated group (Figure 2). However, no significant difference was observed in LH level in 5 mg/kg TQ when compared with the control.

There was a significant decrease ($p < 0.05$) in Follicle stimulating hormone (FSH) in PbA, PbA+ 3.75 mg/kg TQ, PbA + 2.5 mg/kg TQ when compared with control. There was no FSH in PbA + 5 mg/kg TQ and 5 mg/kg TQ when compared with the control. A significant increase ($P < 0.05$) in the level of FSH was observed in PbA+ 5 mg/kg TQ and 5 mg/kg TQ when compared with PbA-treated groups. However, no significant difference in FSH levels in PbA+3.75 mg/kg TQ and PbA + 2.5 mg/kg TQ when compared with PbA-treated groups (Figure 3).

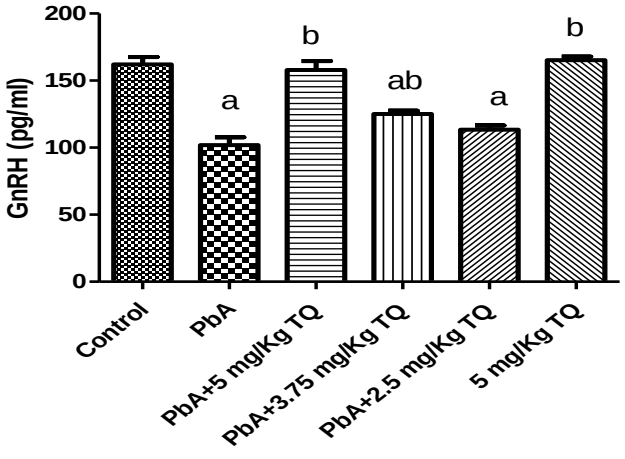


Figure 1: Effects of thymoquinone (TQ) on Gonadotropin-releasing hormone (GnRH) in lead acetate-induced reproductive toxicity in male Wistar Rats.
a: significant decrease ($p < 0.05$) when compared with the control group.
b: significant increase ($p < 0.05$) when compared with PbA treated group.

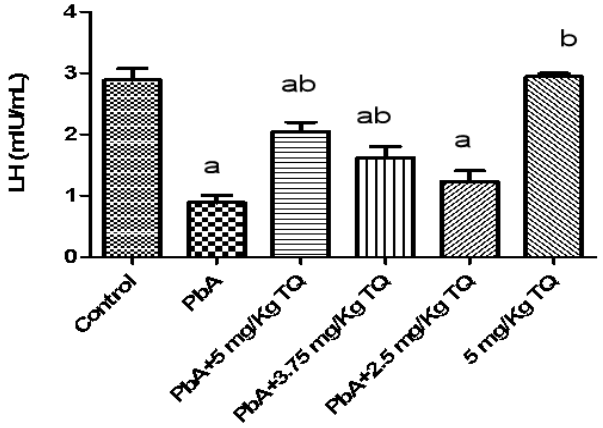


Figure 2: Effect of thymoquinone (TQ) on Luteinizing hormone (LH) in lead acetate-induced reproductive toxicity in male Wistar Rats.
a: significant decrease ($p < 0.05$) when compared with the control.
b: significant increase ($p < 0.05$) when compared with pbA treated group

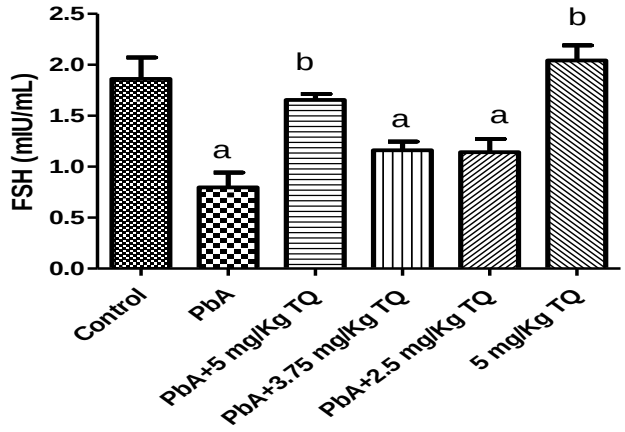


Figure 3: Effects of thymoquinone (TQ) in Follicle stimulating hormone (FSH) on lead acetate-induced reproductive toxicity in male Wistar Rats.
a: implies a significant decrease ($p < 0.05$) when compared with the control.
b: implies a significant increase ($p < 0.05$) when compared with PbA-treated groups.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

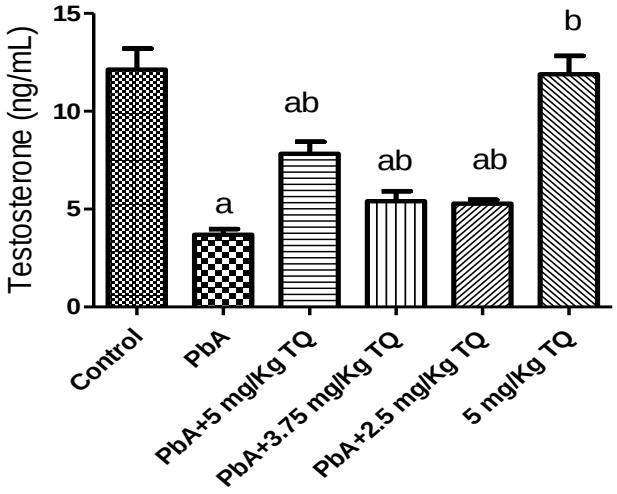
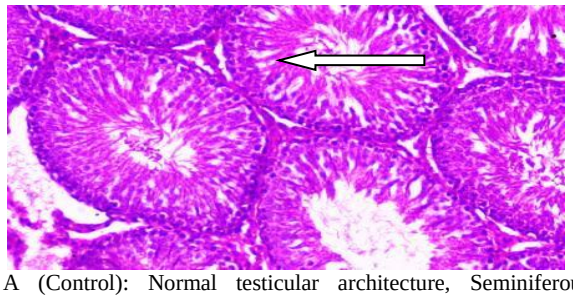


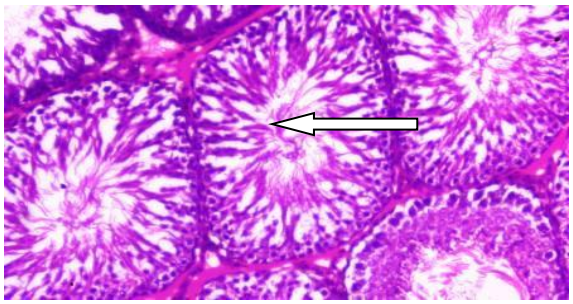
Figure 4: Effects of thymoquinone (TQ) on Testosterone in lead acetate-induced reproductive toxicity in male Wistar Rats.
a: implies a significant decrease ($p < 0.05$) when compared with the control.
b: implies a significant increase ($p < 0.05$) when compared with PbA group

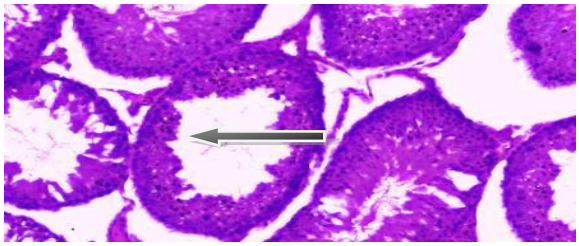
There was a significant decrease ($P < 0.05$) in Testosterone in PbA, PbA + 5 mg/kg TQ, PbA+3.75 mg/kg TQ, PbA + 2.5 mg/kg TQ when compared with control groups. However, testosterone in PbA+ 5 mg/kg TQ, PbA+3.75 mg/kg TQ, PbA + 2.5 mg/kg TQ, 5 mg/kg TQ treated groups significantly increased when compared with PbA treated groups (Figure 4).
The mechanisms through which Pb2+ causes reproductive damages are oxidative stress, decrease antioxidant levels, direct toxic effects on sperm cells, and alteration in the hypothalamic–pituitary–testicular axis.[32,33,34] Thymoquinone has been reported to improve sexual hormones9 and reproductive characteristics.[11]

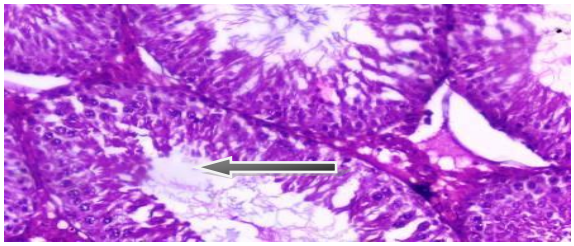
Serum, GnRH, testosterone FSH, and LH analyzed in this study showed significant reduction. This is in line with the report of Sokol *et al*.,[35] and Alawa *et al*.[36] Administration of Thymoquinone ameliorated all the hormonal insufficiencies caused by PbA. The decreased serum FSH level may be attributed to the deleterious action of PbA on the pituitary-testis axis, causing hormonal disruption and the modulation of Sertoli cell function such as the depletion of Sertoli cell numbers or disruption of blood-testis-barrier,[37] which in turn may disrupt spermatogenesis.[38] Studies suggest that the decreased LH level is due to Pb-induced disorders in the hypothalamic control of pituitary hormone secretions, which depicts a disruption in the gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus and disrupting LH release.[21] Together with FSH and LH, testosterone is necessary for the development, growth, and normal functioning of the testes and the secretory activities of the reproductive organs.[39] The secretion of testosterone by Leydig cells is dependent upon the secretion of Luteinizing hormone (LH) by the pituitary gland.[40] Testicular function and the male reproductive system are almost entirely controlled by testosterone.[41]

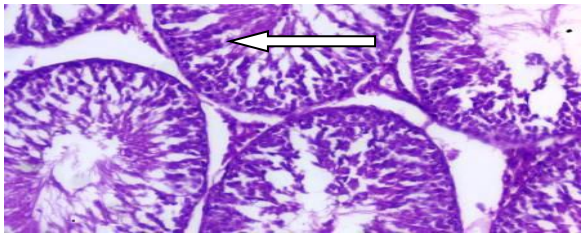
Effects of Thymoquinone on Testicular and epididymal histology in Lead acetate-Induced Reproductive Toxicity in Male Wistar Rats
Histopathological studies of the testis in groups C, D, and F show no effect of PbA-induced repro-toxicity when compared with control while groups B and E show moderate and severe maturation arrest in the seminal tubules respectively when compared with control (Figure 5). Epididymal histology shows a loosely packed epididymal duct with empty spermatozoa in groups B and E when compared with the control (Figure 6).
Histopathology studies showed germinal epithelial depletion of the seminiferous tubule with maturation arrest as well as loosely packed epididymal ducts with empty luminal spermatozoa. This was reversed by TQ treatment.
Studies have substantiated that thymoquinone's potent antioxidant properties are capable of scavenging reactive oxygen species (ROS).[42] Amelioration of hypothalamic pituitary testicular hormones negatively affected by PbA in this study is an indication that thymoquinone is capable of stimulating the hypothalamus and Leydig cells in the testis for the production of GnRH and testosterone respectively.


A (Control): Normal testicular architecture, Seminiferous tubules, and normal maturation stages with the presence of spermatozoa within their lumen (White arrow). (H &E) ×400

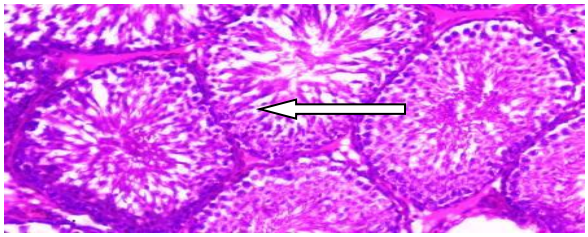

D (PbA + 3.75 mg/Kg TQ): Normal testicular architecture, seminiferous tubules and maturation stages. (H &E) ×400


B (PbA): Few seminiferous tubules showing maturation arrest (Black arrow) (H &E) ×400


E (PbA + 2.5 mg/Kg TQ):Few seminiferous tubules showing maturation arrest (Black arrow) (H &E) ×400

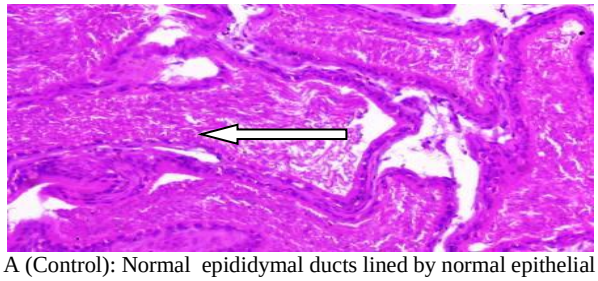


C (PbA +5mg/Kg TQ): Normal testicular architecture, Seminiferous tubules, and maturation stages. (H &E) ×400

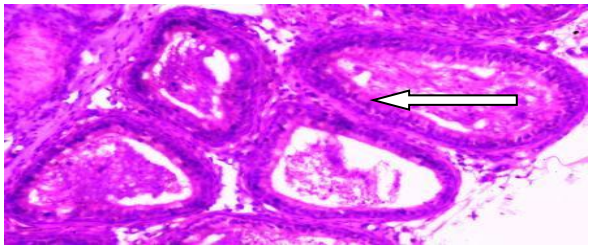


F(5 mg/KgTQ): Normal testicular architecture, Seminiferous tubules and maturation stages (H &E) ×400

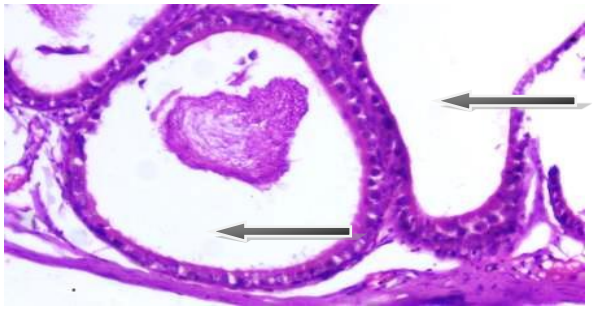
Figure 5: Photomicrograph of Testicular Section



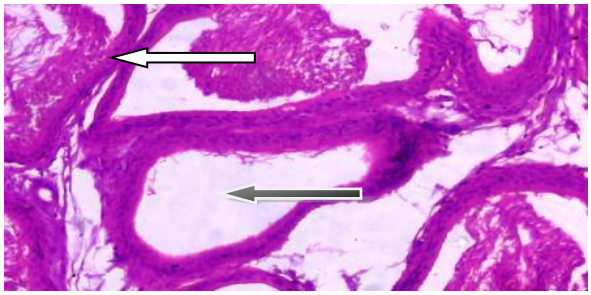
A (Control): Normal epididymal ducts lined by normal epithelial and smooth muscle layer, the ducts are seen storing spermatozoa within the lumen (white arrow) (H & E) ×400



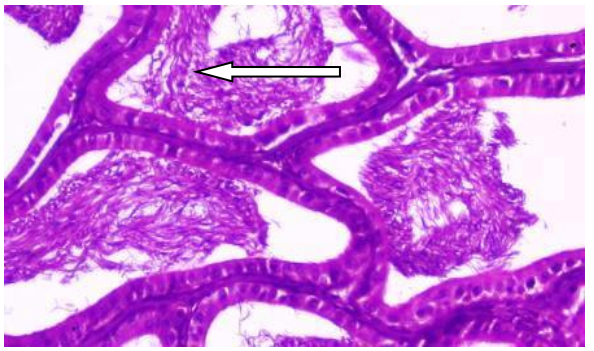
D (PbA + 3.75 mg/Kg TQ):Normal epididymal ducts with luminal spermatozoan storage(White arrow) (H &E) ×400



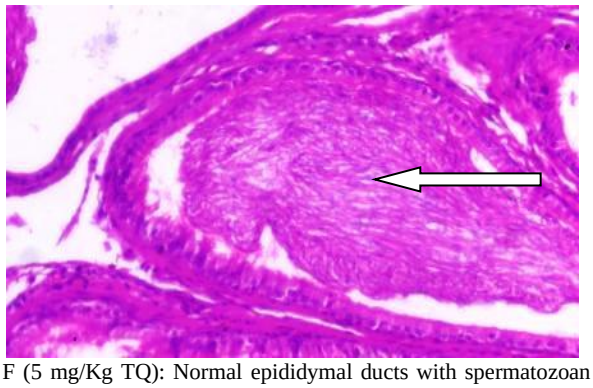
B (PbA): ducts with empty luminal spermatozoa (Black arrow) (H & E) ×400



E (PbA + 2.5 mg/Kg TQ): Loosely packed ducts (White arrow) with empty luminal spermatozoa (Black arrow)(H & E) ×400



C (PbA + 5 mg/Kg TQ): Normal epididymal ducts with luminal spermatozoan storage (White arrow) (H &E) ×400



F (5 mg/Kg TQ): Normal epididymal ducts with spermatozoan storage (White arrow) (H &E) ×400

Figure 6: Photomicrographs of Epididymal Section

Conclusion

Thymoquinone ameliorated the reproductive toxicity induced by lead acetate in male Wistar rats, suggesting thymoquinone is a possible therapeutic agent in improving male reproductive functions in PbA toxicities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The technical support of Miss M.A.M Awonuga, Mrs. M.A Salaam, and Mr D. Osuolale of the Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria is gratefully acknowledged.

References

1. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdisciplinary toxicology. 2012; 1;5(2):47-58.
2. Osmel LL, José MSP. Effects of lead on Reproductive Health. Lead chemistry. 2020; 3-20, London. Intech Open.
3. Santhosh KR, Asha DS. Lead Toxicity on Male Reproductive System and its Mechanism: A Review. Research J. Pharm. and Tech. 2018; 11(3): 1228-1232.
4. Mustari A, Nooruzzaman M, Miah M A, Sujan K M, Chowdhury E H. Promoting action of vitamin E and black seed oil on reproductive hormones and organ histoarchitecture of Swiss albino mice. Vet. Med. and Sci, 2022; 8:710–718.
5. Asiyah HA, Syazana NS, Hashida NH, Durriyyah SHA, Kamaruddin MY. Effects of nicotine and gelam honey on testis parameters and sperm qualities of juvenile rats. Sci Res Essays. 2011; 6(26):5471-5474.
6. Vijayakumar S, Divya M, Vaseeharan B, Chen J, Biruntha M, Silva LP, Dasgupta N. Biological compound capping of silver nanoparticle with the seed extracts of blackcumin (Nigella sativa): a potential antibacterial, antidiabetic, anti-inflammatory, and antioxidant. J. Inorg. & Organo. Poly. & Mat., 2021; 31, 624-635.
7. Bordoni L, Fedeli D, Nasuti C, Maggi F, Papa F, Wabitsch M. Gabbianelli R. Antioxidant and anti-inflammatory properties of *Nigella sativa* oil in human pre-adipocytes. Antioxidants, 2019; *8*(2), 51.
8. Darand M, Mirmiran P, Mokari-Yamchi A. The effect of *Nigella sativa* on infertility in men and women: A systematic review. Prog in Nut. 2020; 21:33–41
9. Haseena S, Manjunath A, Kusal K D, Shaik HS. Effects of Thymoquinone on Testosterone and LH levels in Streptozotocine Induced Diabetic male Albino Rats.J. Pharm. Sci. & Res. 2015; 7(8), 554-556.
10. Sayeed MSB, Shams T, Hossain SF, Rahman MR, Mostofa AGM, Kadir MF, Mahmood S, Asaduzzaman M *Nigella sativa* L. seeds modulate mood, anxiety and cognition in healthy adolescent males. J Ethnopharmacol 2014; 152:156-162.
11. Al-Zahrani S, Mohany M, Kandeal S, Badr G. Thymoquinone and vitamin E supplementation improve the reproductive characteristics of heat-stressed male mice. J Med Plants Res. 2012; 6(3): 493-499.
12. Rosli FD. Ameliorative Effects of Thymoquinone on Sperm Parameters and Testosterone Level of Nicotine-Treated Sprague Dawley Rats. Braz. Arch. Bio. & Tech. 2019; ISSN 1678-4324.
13. Ghosh DE, Firdaus SB, Mitra EL, Chattopadhyay AI, Pattari SK, Jana KU, Bandyopadhyay DE. Ameliorative effect of curry leaf aqueous extract against lead acetate-induced oxidative stress in rat kidneys. Int J Pharm Pharm Sci. 2013;5(4):546-56.
14. Tekeoglu I, Dogan A, Ediz L, Budancamanak M, Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytotherapy Research: An Int. J. Devoted to Pharmacol. & Toxicol Eval. of Nat. Pro. Deriv. 2007 Sep;21(9):895-7.
15. Prasad R, Brodie G, Vanderwoude C, Hodge S. Potential of the weed *Abrusprecatorious Linnaeus* (Fabale: Fabaceae) for control of insect pests in the south pacific: A review. Int J. Entomo Res. 2016; 3(3):113- 124
16. World Health Organization. World Health Organization laboratory manual for the examination and processing of human semen. World Health Organization: Geneva, Swtizerland. 2010;287.
17. Seed J, Chapin RE, Clegg ED. Methods for assessing sperm motility, morphology and counts in rat, rabbit and dogs: A consensus report. Reprod. Toxicol. 1996; 10237-244.
18. Adedokun KI, Akande AT, Osuntokun OS, Oladokun OO, Akintayo CO, Atere TG. Evaluation of the Markers of Reproductive Functions in the Male Wistar Rats Treated with Analytical Grade Glyphosate. Trop. J. Health Sci. 2022 Aug 4;29(2):20-6.
19. Gandhi J, Hernandez R, Chen A, Smith N, Sheynkin Y, Joshi G, Khan S. Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. Zygote, 2017; 25(2), 103-110. doi:10.1017/S0967199417000028
20. Winder C. Reproductive and chromosomal effects of occupational exposure to lead in the male. Reprod. Toxicol. 1989; 3:221-233.
21. Sokol RZ, Wang S, Wan YJ, Stanczyk FZ, Gentzschein E, Chapin RE. Long-term, low-dose lead exposure alters the gonadotropin-releasing hormone system in the male rat. Environ Health Perspect. 2002; 110:871–874.
22. Wadi SA, Ahmad G. Effects of lead on the male reproductive system in mice. J Toxicol Environ Health. 1999; 56(7):513-21.
23. Raji Y, Akinsomisoye OS, Salman TM. The antispermatogenic activity of Morinda lucida extracts in male rats. Asian J. androl; 2005; *7*(4): 405-410.
24. Zhou YM, Zheng Q, Shi L, Zhang W, Zhen W, Chen YL. An epididymis-specific secretory protein HongrES1 critically regulates sperm capacitation and male fertility. PloS one. 2008; 3(12)4106: 1-12
25. Graca A, Ramalho-Santos J, de Lourdes PM. Effect of lead chloride on spermatogenesis and sperm parameters in mice. Asian J Androl; 2004; 6: 237-241.
26. Kasperczyk S, Birkner E, Kasperczyk A, Zalejska-Fiolka J. The activity of superoxide dismutase and catalase in people protractedly exposed to lead compounds. Ann Agric Environ Med. 2004; 11:291–296
27. Benoff S, Centola GM, Millan C, Napolitano B, Marmar JL, Hurley IR. Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. Human Reprod. 2003; 18:374-383.25.
28. Yulianto RA, Isnaeni W, Susanti R. Pengaruhpemberian vitamin E terhadap kualitas sperma tikusputih yang dipapar timbal. Unnes J. Life Sci. 2013; 2 2.
29. Marianto A, Mahatmanti FW. Acta Scientiarum. Bio. Sci 2020; 401.
30. Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF. Effects of Nigella sativa L. seed oil on abnormal semen quality in infertile men: a randomized, double-blind, placebo-controlled clinical trial. Phytomedicine. 2014 15;21(6):901-5.
31. Mahdavi R, Javad H, Nazli N. Effects of black seeds (*Nigella sativa*) on male infertility. A systematic review. J. Herbal Medicine. 2015; 5(3):133-139
32. Pandya C, Pillai P, Nampoothiri LP, Bhatt N, Gupta S, Gupta S. Effect of lead and cadmium co-exposure on testicular steroid metabolism and antioxidant system of adult male rats. Andrologia 2012; 44:813–822.
33. Oyeyemi AW, Anyanwu CP, Akinola AO, Daramola OO, Alli OB, Ehiaghe FA. Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. JBRA Assist Reprod 2019; 23(4):336343
34. Oyeyemi WA, Daramola OO, Akinola AO, Idris AO, Aikpitanyi I. Hepatic and reproductive toxicity of sub-chronic exposure to dichlorvos and lead acetate on male Wistar rats. Asian Pacific J Reprod 2020; *9:283–290*

35. Sokol RZ. Hormonal effects of lead acetate in the male rat: Mechanism of action1. Bio. of Reprod. 1987; 37:1135-1138.
36. Alawa ZM, Al-Omary HL, Jaafar I. Environmental lead exposure and male infertility. IOSR J Dent and Med Sci. 2016; 15:49-54.
37. Siu ER, Mruk DD, Porto CS, Cheng CY. Cadmium-induced testicular injury. Toxicol Appl Pharmacol. 2009; 2382:240–249.
38. Wang X, Wang M, Dong W, Li Y, Zheng X, Piao F, Li S Subchronic exposure to lead acetate inhibits spermatogenesis and down regulates the expression of Ddx3y in testis of mice. Reproductive. Toxicol. 2013; 42:242–250.
39. Weinbauer GF, Luetjens CM, Simoni M, Nieschlag E. Physiology of Testicular Function. Andrology. Springer, Berlin, Heidelberg. 2010; 11-59.
40. Uzun FG, Demir FS, Kalender HB, Kalender Y. Protective effect of cat-echin and quercetin on chlorpyrifos-induced lung toxicity in male rats. *Food* Chem Toxicol 2010; 48:1714–1720.
41. Daoud S, Tahri A, Rim K, Kamilia K, Tahia B, Zouheir S. Safety assessment of cefuroxime on male reproductive system: Sub-acute toxicity and potential reversibility after a complete cycle of spermatogenesis and epididymal maturation in Wistar rats. Reprod. Toxicol. 2019; 85: 75-82.
42. Al-Naqeep G, Ismail M, Chan KW. *Nigella sativa* Thymoquinone rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. Free Radic Biol Med. 2012; 48: 664-72.