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Physiological Therapeutic Protective Function of Thymoquinone on Mice Fertility

Jabbar Jassim Hamady[1], Mohammed S. A. Hayyawi[1]

[1]*Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq*

**Corresponding Author: Jabbar Jassim Hamady*
Email: jhamady@uowasit.edu.iq

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Abstract

The experiment was conducted in the college of veterinary medicine at the University of Wasit to determine the role of therapeutic administration of thymoquinone on male mice fertility as a muddle for mammalian. Through the study, 16 mice have been separated into two groups equally: the control group administered distal water and kept in 25±2ºC, and the thymoquinone groups kept at 25±2ºC and administered thymoquinone 25 mg/kg b.w. for 2 weeks. After the end of the drenched period on the last day of the experiment, mice were not drenched for 24 hours, and ketamine 100 mg/kg and xylazine 10 mg/kg were administered to the scarified mice. 100 mg of testis samples were taken from the testis of all groups and kept at liquid nitrogen for PCR to determine the effects of stress through significant differentiation of HSP70 protein in testis tissue. The experiment showed a highly significant effect of the thymoquinone group (25 mg/kg b.w.) when compared with the control group.

Introduction

The protective effects of thymoquinone are anti-toxicity, anti-inflammatory, antioxidant, anti-apoptosis, autophagy, immunomodulatory, anti-hypertensive, and regulate the apoptotic pathway (Atta et al., 2017; Attari et al., 2018; Yaghutian Nezhad et al., 2021; Adana et al., 2022; Shojaedini et al., 2024). On the other hand, more research has referred to the role of thymoquinone as an ability mechanism against many diseases caused by bacteria, viral, fungal, and protozoa (Elsharkawy et al., 2021; Fatima Shad et al., 2021; Qureshi et al., 2022; El-Sayed and Rizk, 2023; Nouri et al., 2023). So, the researchers confirmed the role of activity and cellular protective effects as antioxidants to protect the cell from apoptosis and autophagy by regulating the antioxidant system, especially glutathione (Akpinar et al., 2023; Isaev et al., 2023; Shahrajabian and Sun, 2024).

The studies have demonstrated the ability of thymoquinone to protect the kidney from damage leading to renal failure and the protective role of endothelial blood vessels in hypocholesteremia. This finding by decrement effect of MDA and increment of glutathione and role protect by reducing cardiotoxicity (Yıldırım et al., 2023; Wardani 2024; Karim et al., 2024). In addition, the activity role of thymoquinone in modulating blood glucose and lipid profile refers to its protective effect on hepatocytes and beta cells of pancreases from damage (Ashour et al., 2023; Caglar et al., 2024; Hafez et al., 2024). On the gastric ulcer, researchers pointed to the effective and influential role of thymoquinone ability to protect gastric mucosa from gastric acid by reducing gastric acid and stmulating gastric mucosa formation, these mechanisms enhancement protective effect of thymoquine as antiulcer (Khan et al., 2023; Madani et al., 2023; Tiwari et al., 2024)

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Showed the result of studies on reproductive mammalian system effectivity role of thymoqynone to downregulation of MDA , and enhancement level of glutathione, SOD, TAS and nitric oxide on ovarian follicles and testicular tissues , these mechanisms give important role of thymoquinone to protective ability effects on reproductive system from infertility caused by apoptosis and autophagy result from imbalance of pro-oxidant/antioxidants and apoptotic pathway (Sayed et al., 2014; Hassan et al., 2019; Saha, et al., 2021; Shojaedini et al., 2024). In addition to the role of thymoqunone protective spermatogenesis formation from effect toxicity, heat stress, and hypoxia, these mechanisms refer to the effects of thymoquinone on Sertoli cell and Leydig cell function to regulate spermatogenesis through release factors inhibin, androgen binding protein, and release enzyme aid to convert testosterone to estrogen and to 5α-dihydrotestosterone (Hassan et al., 2019; Gur et al., 2021; Khalil et al., 2023). Studies explained the activity role of thymoqunone on hypothalamus and pituitary function through enhanced secretion of hormones, especially FSH and LH, that are responsible for spermatogenesis (Ghahremani & Mahmoudi, 2022; Manoharan et al., 2024).

Methods

Experimental Design and Animal Grouping

The sample for this study comprised of sixteen mature male mice; all the mice were sixty-five days of age with an average weight of thirty-two ± five grams. Specifically, the animals chosen provided minimal variation in physiological parameters that would otherwise affect the results from the study. The mice were divided into two groups with equal number of eight individuals chosen at random to make the distribution of subjects manageable. Environmental factors were kept constant in the research experiment conducted in the College of Veterinary Medicine, University of Wasit; the mice were placed an environment under a maintained ambient temperature of 25 ± 2°C. This stable temperature setting was very usefulual in excluding various external factors that could affect the physiology of the test subjects and thus would in turn affect the outcomes of thymoquinone.

In the grouping protocol the first group, the Control Group (C), received distilled water vehicle control to a comparative level. This control made it possible to compare the experimental group with the administration of thymoquinone and any observed effects from other procedural variables would be canceled out. The second group was labeled the Thymoquinone Group (TQ); these animals received a controlled daily dose of thymoquinone at a volume of 25mg/kg body weight. The dose was chosen according to the findings of previous investigations of the similar physiological experiments, and was given in single injections lasting for two weeks consecutively. The duration was selected in order to assess the anti-oxidative and protective effects of thymoquinone on male fertility under experimental models of environmental stressors.

Thymoquinone Administration and Sample Collection

To avoid dosing errors, thymoquinone was orally administered daily to the TQ group using standard laboratory techniques that help to avoid situations of either over dosing or under dosing the animals. It extended for 14 days, which is consistent with other experimental paradigms applied to measure acute effects of therapeutic agents on physiological processes. At the end of the two week treatment regimen, the final dose was administered and the mice left untreated for 24 hours for any metabolic or transient effects of thymoquinone to en-shroud themselves.

After this time, humane sacrifice was carried out as to gather tissue samples.In. Surgical anaesthesia was accomplished intraperitoneally using ketamine hydrochloride at a dose of 100

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mg/kg body weight and xylazine at 10 mg/kg body weight. After the animals were completely under the influence of anesthesia, a surgical biopsy of testicular tissue was performed. In each of the subjects, 100mg of testicular tissue was taken and immediately stored in liquid nitrogen. This preservation technique is important in order to preserve RNA integrity and proteins present in the samples especially when analysing the HSP70 protein level as a marker for cellular stress. This is done to ensure that the rate of degradation is slowed down and measured systematically at later phases of molecular testing carried out under liquid nitrogen.

Statistical Analysis

To compare means of the data collected between control and treatment groups a post test was performed by GraphPad Prism software. One- way ANOVA tests were used to determine the significance of values between the two groups with a 5% level of significance. This test is used when making comparison on two groups and checking for those differences which indicate that there is a real difference and that there is an impact of the thymoquinone treatment. The selected p-value of 0.05 is statistically acceptable whereby a higher risk of type I errors that give impression of significance where there really isn't is minimized.

The primary biomarker chosen for this study was to measure the HSP70 level within testis tissue, because HSP70 can be used as a primary biomarker to estimate the response to the tissue stress. Association of higher levels of HSP70 are again favourable as this protein is involved in maintaining proteins that are vulnerable when the cell is under stress, prevents apoptosis and can act as a repair factor in such cellular stress. Thus, by measuring HSP70 in testicular tissues, we intended to assess whether thymoquinone offers considerable protection in orchitis models to enrich the knowledge for fertility preservation. The information collected from these assessments shed light on how this compound work including its role in enhancing cell protection and reproductive capacity.

Results and Discussion

By dividing the sample's optical density (260) by its optical density (280), the normalized RNA optical density should be less than 2.1 and greater than 1.8 (Figure 1). The result of the RNA concentration of heat shock protein in (Figure 2) between groups showed a highly significant difference ($p<0.05$) between the control group and the treatment group of thymoquinone.

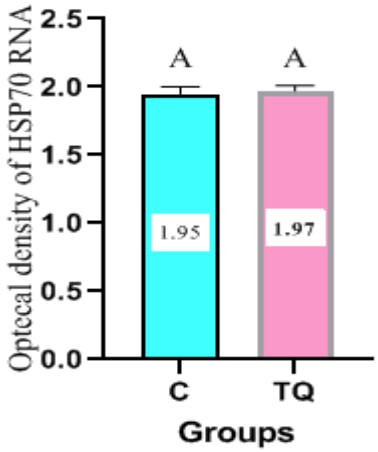

Figure 1. Effect of thymoquinone on testis tissue optical density of HSP70 RNA in adult male mice after 2 weeks.

The heat shock protein gene expression results in (Figure 3) between treatment and control groups in the experiment revealed a highly significant ($p<0.05$) effect with thymoquinone 25

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mg/kg b.w. provided to the treatment group. When compared to the control group, the thymoquinone group's increase in heat shock protein fold gene expression had a significant ($p<0.05$) impact.

C= Control groupwas kept at 25±2°C for 2 weeks and drenched distal water; TQ= thymoquinone group was kept at 25±2°C for 2 weeks and drenched at 25 mg/kg b.w. for 2 weeks.

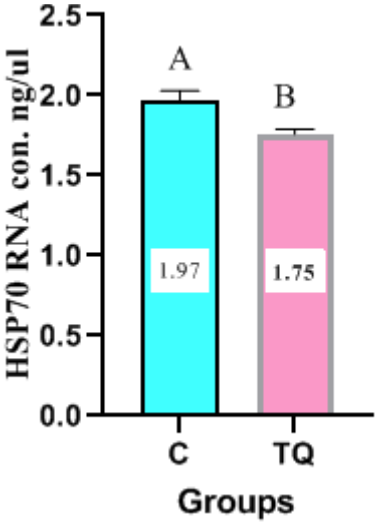

Figure 2. Effect of thymoquinone on testis tissue RNA concentration of HSP70 in adult male mice after 2 weeks.

C= Control group was kept at 25±2°C for 2 weeks and drenched distal water; TQ= thymoquinone group was kept at 25±2°C for 2 weeks and drenched at 25 mg/kg b.w. for 2 weeks.

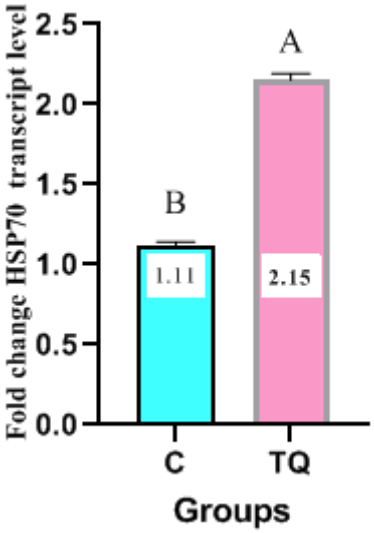

Figure 3.: Effect of thymoquinone on testis tissue gene expression of HSP70 in adult male mice after 2 weeks.

C= Control group was kept at 25±2°C for 2 weeks and drenched distal water; TQ= thymoquinone group was kept at 25±2°C for 2 weeks and drenched at 25 mg/kg b.w. for 2 weeks.

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The experiment explained the protective mechanism of thymoquinone on mice's testis fertility through an enhancement fold of heat shock protein expression that resulted in protection of cell activity and enhancement of spermatogenesis formation by activation of Sertoli cell and Leydig cell functions that are responsible for hormone secretion, inhibin, androgen binding protein, and testosterone. These mechanisms referred to the effects of thymoquinone on mice's testis fertility (Hassan et al., 2019; Gur et al., 2021; Khalil et al., 2023; Soliman et al., 2024). The role of thymoquinone on testis fertility is enhanced by the enhanced fold activity of heat shock protein 70, which plays a protective role on testicular cells by activating the antioxidant system and regulating the apoptotic pathway that keeps and activates cellular enzyme activity (Saha et al., 2021; Shojaedini et al., 2024). On the other hand, regulation of hypothalamus and pituitary glands by thymoquinone involves these mechanisms: activation of thymoquinone to the inhibin and testosterone that have a positive effect on hypothalamus and pituitary glands when decreased hormone secretion (Gur et al., 2021; Khalil et al., 2023; Nozad et al., 2024).

Stimulation fertility by enhancement secretion of GnRH, FSH, and LH hormones by thymoquinone that give mechanism stimulation responses on sperm quality, motility, and sperm count (Alaee et al., 2023; Yalçın et al., 2024). So, administration of thymuqinone to the rodents increases cellular activity of the testis through decreased levels of MDA, dead sperm, and abnormal sperm, while increasing glutathione, SOD, and nitric oxide (AlGaradi et al., 2023; Khalil et al., 2023). the result of the experiment confirmed that the administration of thymoquinone increased the gene expression of heat shock protein in the testis tissues when compared to the control.

Conclusion

These results concluded the protective effects of thymoquinone on testicular cell activity by enhancing the enzymes and hormones responsible for spermatogenesis formation and spermatozoa quality through increased sperm count, motility, sperm PH, and decreased abnormal sperm All these mechanisms referred to increased fertility by thymoquinone administration.

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