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Effect of stress environmental conditions on mice fertility

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ABSTRACT

The study was conducted in the college of veterinary medicine at the University of Wasit to determine the role of heat stress on mammals. Through the study, 24 mice have been separated into three groups equally: the control group administered distal water and kept in 22±2ºC, stressed mice kept at 32±1ºC and administered distal water for 2 weeks, and thymoquinone groups kept at 32±1ºC and administered thymoquinone 30 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 (30 mg/kg b.w.) when compared with the stressed group kept at 32±1ºC.

1. Introduction

Heat stress causes loss of fertility in mice after two weeks, approximately when kept at 32±1°C (Cammack et al., 2009; Rizzoto et al., 2020). The major factor affecting animal reproduction, known as heat stress, is that the temperature of the testis should be below the body temperature, less than 3–5ºC (Rizzoto et al., 2020). So, Rizzoto et al. (2020) referred to the effect of heat stress on spermatogenesis production by decreasing the antioxidant system, apoptotic pathway, and heat shock protein, and the effect of a testicular temperature increment, which causes a reduction in sperm motility and morphology due to hypoxia without increasing testis tissue blood flow. The main stress factors that cause a reduction in the spermatogenesis mechanism result from an increase in testis temperature, as heat stress should be avoided to prevent apoptosis, autophagy, and DNA damage (Costa et al., 2018). These result from an imbalance mechanism regulating the testis antioxidant system and apoptotic factors (Durairajanayagam et al., 2015; Costa et al., 2018; Aldahhan and Stanton, 2021). Exposed animals to higher environmental temperatures cause a change in sperm morphology, an alteration in testicular mass, and a decrease in testosterone levels (Costa et al., 2018). Heat stress decreased sperm motility by 97%, sperm count by 42%, and disrupted blood-testis barrier integrity. On the other hand, it reduced testis retinol concentration by 23% while increasing serum retinol concentration 25% (Aldahhan and Stanton 2021; Cao et al., 2023).

Thymoquinone has biological activity as an antioxidant, an anti-inflammatory agent, an antihypertensive, and has protective effects against heat stress, male reproduction from various harmful conditions, spermatogenesis damage, and epididymis sperm count toxicity (Hassan et al., 2019). These mechanisms are regulated by thymoquinone through the hypothalamus-pituitary testis axis, and responses are effected by stimulation of the testis. Pro-oxidant antioxidant balance has anti-apoptotic effects (Hassan et al., 2019). Thymoquinone treatment enhances sperm quality and serum hormone levels (LH, FSH, and testosterone). These mechanisms act as therapeutic protective effects against infertility caused by apoptosis and autophagy (Radad et al., 2014; Hassan et al., 2019; Nazir et al., 2022; AlGaradi et al., 2023).

2. Methodology

Ethical Approval

The study was approved by The Scientific Committee of the College of Veterinary Medicine in the University of Wasit.

Experimental design

Twenty-four mature male mice were randomly divided into three groups, each containing eight mice, for two weeks. Control group (C) kept in 22±2ºC and administered distal water for two weeks; stressed group (ST) stressed mice kept at 32±1ºC and administered distal water for 2 weeks; thymoquinone group (Q) mice kept at 32±1ºC and administered thymoquinone 30 mg/kg b.w. for 2 weeks. At 24 hours after late treatment, mice were administered ketamine 100 mg/kg b.w. i.p. and xylazine 10 mg/kg b.w. i.p. for anesthesia and scarification. 100 mg of each sample from the testis kept in liquid nitrogen for HSP70 expression. RNA isolation according Danga and Rath (2024).

Statistical analysis

The result of the experiment was analyzed by graph pad prism program compression performed using the one-way ANOVA to detect significant differences between study groups at $p< 0.05$ (Gharban and Yousif, 2021).

3. Results and Discussion

Normalization of RNA to determine optical density by dividing optical density 260 of the sample by optical density 280, the result should be less than 2.1 and more than 1.8 (Figure 1). The result of the RNA concentration of heat shock protein among groups showed a highly significant difference ($p<0.05$) between the control group and the stressed group. Treatment group of thymoquinone showed highly significant ($p<0.05$) with stressed group, while nonsignificant between control group and treatment group of thymoquinone.

The result of heat shock protein gene expression among groups of experiment showed highly significant ($p<0.05$) effect between treatment group administered thymoquinone 30 mg/kg b.w. under heat stress (32±1ºC) and stressed group kept at 32±1ºC. The increment of gene expression of heat shock protein fold in the thymoquinone group showed a significant ($p<0.05$) effect when compared with the control group, while there was a slightly significant ($p<0.05$) difference between the control and stressed groups.

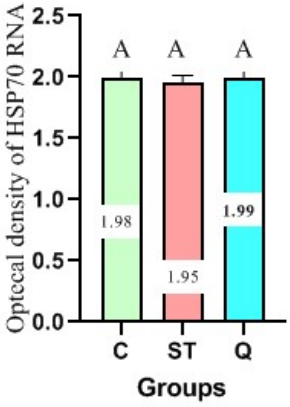

Figure 1 Effect of heat stress on testis tissue optical density of HSP70 RNA in adult male mice after 2 weeks
C= Control group drenched distal water. ST = stressed group kept at 32±1ºC for 2 weeks and drenched in distal water. Q= thymoquinone group was kept at 32±1°C for 2 weeks and drenched at 30 mg/kg b.w. for 2 weeks.

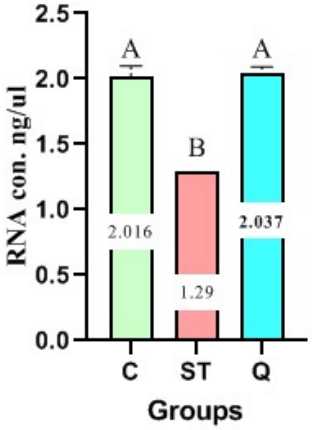

Figure 2: Effect of heat stress on testis tissue RNA concentration of HSP70 in adult male mice after 2 weeks
C= Control group drenched distal water. ST = stressed group kept at 32±1ºC for 2 weeks and drenched in distal water. Q= thymoquinone group kept at 32±1°C for 2 weeks and drenched at 30 mg /kg b.w. for 2 weeks.

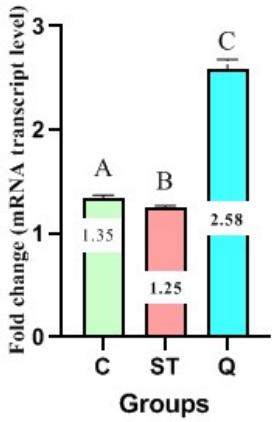

Figure 3: Effect of heat stress on testis tissue gene expression of HSP70 in adult male mice after 2 weeks
C= Control group drenched distal water. ST = stressed group kept at 32±1ºC for 2 weeks and drenched distal water. Q= thymoquinone group kept at 32±1°C for 2 weeks and drenched at 30mg /kg b.w. for 2 weeks

The experiment referred to the protective therapeutic effect of thymoquinone against heat stress, which causes a lower quality of testicular cell production, especially Sertoli cells and Leydig cells. These mechanisms lead to the therapeutic activity of thymoquinone by decreasing the effect of heat stress and enhancing the cellular activity of the testis through the reduction effect of apoptosis and autophagy, which result from an imbalance of pro-oxidant/antioxidant, apoptotic pathways, and heat shock protein as indicators in the experiment (Al-Zahrani et al., 2012; Mabrouk et al., 2018; Hassan et al., 2019; Öztürk et al., 2020; AlGaradi et al., 2023; Ibrahim et al., 2023). The study showed effect of heat stress on testicular function through keeping adult male mice at 32±1°C for 2 weeks. Mice protocol stress lead to disturbances in the testis cellular function by downregulation of the antioxidant system and upregulation of the apoptotic pathway, leading to an increment of apoptosis and autophagy. These mechanisms downregulate Sertoli cell and Leydig cell quality function to control regulation of testicular fertility (Rizzoto et al., 2020; Shahat et al., 2020; Dang-Cong and Nguyen-Thanh, 2022; Gao et al., 2022; Gan et al., 2022; Yang et al., 2024).

Stressed environmental condition effect on mice fertility through reduction mechanism regulation of hypothalamus pituitary testis axis lead to decrement in hormonal regulation of testis function, especially LH and FSH hormones that regulate Sertoli cell and Leydig cell to secrete testosterone from Leydig cell and inhibin to negative and positive mechanism effect on hupothalamus and pituitary gland to regulate FSH and LH secretion from adenohypophysis (Li et al., 2020; Lu et al., 2021; Kim et al., 2022; Li et al., 2023; Silva et al., 2024; Yang et al., 2024). Administration of thymoquinone orally to the stressed male mice for 2 weeks

gives significant effect by enhancement gene expression of heat shock protein 70 fold when compared with effect of stressed mice drenched distal water, this result referred to protective therapeutic effect of thymoquinone on the testicular cell function to regulate spermatogenesis production through reduction effect of apoptosis and autophagy result from stressed environment condition causes downregulation of heat shock protein 70, these mechanism referred to cellular protective effect of thymoquinone to reduce effect of apoptotic pathway to activation testis cellular function to enhancement fertility by regulate hypothalamus pituitary testis axis function through control secrtion of FSH and LH, that activation Sertoli cell and Leydig cell function to release and production spermatozoa (Adedokun et al., 2023; Alaee et al., 2023; Nozad et al., 2024)

4. Conclusion

The experiment referred to the role of thymoquinone therapeutically protective testicular cell quality against stressed environmental conditions by regulating cellular mechanisms by enhancement of heat shock protein to protect testis cells from apoptosis and autophagy that are increased by stress mice exposure to an increase in average temperature.

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