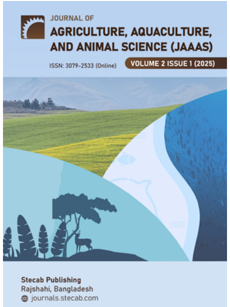

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Research Article

Therapeutic Administration of Thymoquinone on Potential Regulation of Folliculogenesis and Mice Estrus Cycle Activation

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About Article

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ABSTRACT

The study of the evaluation of therapeutic potential administration of thymoquinone on mice estrus cycle function when exposed to the stressed environment condition has been carried out in the animal house at the College of Veterinary Medicine University of Wasit. Thirty mice received three different treatments: The control group received normal saline drenching while group members in the stress and thymoquinone groups received normal saline and ten mg/kg b.w. thymoqunone, respectively. Both groups stayed in (30±1°C) conditions for two weeks. Results indicated that thymoqunone significantly affected reproductive functions at physiological levels than the control group did. The ovaries were collected from all groups post-thymoquinone treatment through synthesized mice which received a mixture of xylazine 10 mg/kg b.w and kitamine 100 mg/kg b.w. The samples were maintained at liquid nitrogen for PCR analysis of stress-induced changes in ovaries tissue through inhibin hormone and HSP70 protein differentiation. The thymoquinone treatment at (10 mg/kg b.w.) created a highly meaningful change compared to the heated (30±1°C) stressed animals for the two-week trial.

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1. INTRODUCTION

Changes of environmental temperature led to an effect on animals' estrus cycle function through change the estrus cycle period by alters the length of the estrus cycle, shorten the period of estrus behavior, and if the temperatures of the environment magnitude, will cause anestrus (Cartwright *et al.*, 2023). The reproductive researcher refers to the role of heat stress on hypothalamus pituitary function ability to release gonadotropin releasing hormone (GnRH) which is important to regulate estrus cycle through endocrine effects on the anterior part of the pituitary gland to secretion follicular stimulating hormone (FSH) and luteinizing hormone (LH) (Yan *et al.*, 2022; Sukhan *et al.*, 2021). The incidences of FSH and LH levels diminished impaired animal reproductive function leading to dysfunction (Gupta *et al.*, 2023). As well as, a stressed environment affects on animals physiological reproductive function through the effect on hypothalamus pituitary gonadal axis potential through impaired secretion of GnRH that stimulate pituitary gland for secretion of FSH and LH to induce endocrine effect on gonads ovary to secretion hormones change of animals behavior lead to initiate of estrus cycle, when impaired this mechanism causes anestrus (Al-Hetty *et al.*, 2023; Ko, 2024).

2. LITERATURE REVIEW

Thymoquinone has pharmacological properties such as antioxidants, antimicrobial, antihistamine, anticancer activities, antifungal and immunomodulatory effects (Neunert *et al.*, 2025; Benjamin, 2025; Abdullaev *et al.*, 2025; Pawar *et al.*, 2025; Okoh *et al.*, 2025). Hamady and Hayyawi (2024a) refers to role of thymoquinone on stressed environment through effect as antioxidant and decreased of heat shock protein expression (Hamady & Hayyawi, 2024a). Studies have proven the role of thymoquinone on hypothalamus activities by increased expression of gonadotropin releasing hormone and causes enhancing of anterior pituitary gland releasing hormones through stimulate gonadotropic cells to the secretion of FSH and LH in the peripheral circulation to stimulate gonad by endocrine effect and enhance behavior properties by appear of clinical signs of animal estrus cycle (Sukatendel *et al.*, 2025; Hamady *et al.*, 2024; Alaee *et al.*, 2023). Thymoquinone might be useful as a protective agent of ovary activities through the improvement of sexual hormones levels in the blood and had putative benefit on ovarian function by amelioration of oxidative stress in the ovary and folliculogenesis (Alaee *et al.*, 2023). Environmental stressed climate effects on healthy mammals through enhancement of free radicals, that cause oxidative cell damage and reduction of apoptotic pathway system led to cell death by apoptosis (Pawar *et al.*, 2025; Okoh *et al.*, 2025; Hamady & Hayyawi, 2024a). The chaperon of heat shock protein 70 (HSP70) as antioxidant function through enhancement of the cellular antioxidant system specially glutathione (GSH), glutathione peroxidase (Gpx), glutathione-s-transferase, SOD, and catalase, these roles important key to protect cell from oxidative stress cause DNA, protein and enzyme damage, and lipid peroxidation (Cartwright *et al.*, 2023; Hamady & Hayyawi, 2024a).

3. METHODOLOGY

3.1. Ethical Approval

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

3.2. Experimental design

Thirty mature female mice underwent random assignment to three separate groups totalling ten animals per group throughout a period of two weeks. Control group (C) kept in (22±1°C) and administered normal saline for two weeks; stressed group (ST) stressed mice kept at (30±1°C) and administered normal saline for two weeks; thymoquinone group (Q) mice kept at (30±1°C) and administered thymoquinone 10 mg/kg b.w. for 2 weeks. 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 ovary kept in liquid nitrogen for HSP70 and inhibin ß expression (Li et al, 2021).

3.3. Statistical analysis

The research data was evaluated through a one-way ANOVA analysis using Graph Pad Prism program processing. The groups demonstrated significant compression with a value of $p \leq 0.05$ (Mohammad *et al.*, 2022).

4. RESULTS AND DISCUSSION

4.1. Result

Figure 1 showcases a significant difference ($p \leq 0.05$) between ovary RNA concentrations of groups exposed to thymoquinone 10mg/kg.bw., and stressed environment (30±1°C) during a two-week experimental period. The figure 2 demonstrates the normalization and quantification of RNA with beta actin to obtain optical density measurements by dividing 280wave with 260 waves which must be within 1.8 to 2.1. Heat shock protein gene expression in ovaries showed a highly significant ($p \leq 0.05$) variation between the thymoquinone-treated experimental group at 10 mg/kg b.w. under heat stress (30±1°C) and the stressed group maintained at (30±1°C) as shown in figure (Sukhan *et al.*, 2021). The thymoquinone-treated group achieved a significant ($p \leq 0.05$) increase in heat shock protein fold gene expression compared to the control group and demonstrated a slightly significant ($p \leq 0.05$) change between the control and stressed groups. The figure 4 displayed the results which revealed that thymoquinone administration in ovary inhibin gene expression produced significant differences ($p \leq 0.05$) versus both the stressed group and control group.

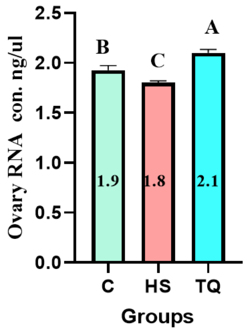

Figure 1. Adult female mouse ovary tissue analysis shows

The above figure shows RNA concentrations under heat stress conditions. The control groups received normal saline drenches. The ovary tissue from adult female mice which received normal saline treatment for two weeks maintained an environment at (30±1°C). This group also underwent drenching with normal saline during testing. The thymoquinone group of mice spent 2 weeks at a temperature of 30±1°C and received 10 mg/kg b.w. of treatment for 2 weeks.

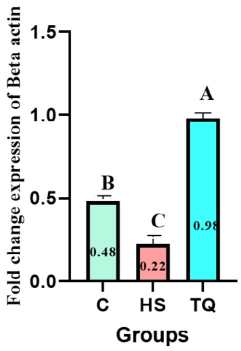

Figure 2. Examined ovary tissue RNA optical density

The study examined ovary tissue RNA optical density in adult female mice after a two-week experimental period using C= Control group that received normal saline while HS = stressed group underwent (30±1°C) treatment for 2 weeks and TQ= thymoquinone group stayed at (30±1°C) and received 10 mg/kg b.w. for 2 weeks. Normal saline water soaked the ovary tissue of mice kept at 30±1°C under stress for 2 weeks. The mouse group receiving 2-week hot stress treatment and 2-week 10 mg/kg body weight drench of thymoquinone was drenched at (30±1°C) for fourteen days.

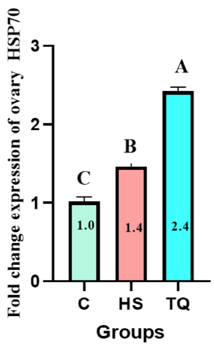

Figure 3. Adult female mice who received normal saline

Figure 3 shows the adult female mice who received normal saline experienced changes in HSP70 gene expression during ovary tissue after exposure to heat stress for 2 weeks in the study groups C and HS. The stressed experimental animals in HS group received (30±1°C) treatment for 2 weeks while being immersed in normal saline. The thymoquinone-treated mice received 10 mg/kg body weight of medication as they remained at 30±1°C for two weeks.

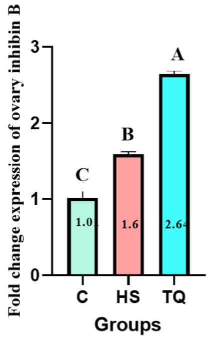

Figure 4. Examined inhibin ß expression at gene level

Figure 4 examined inhibin ß expression at gene level in adult female mouse ovaries after two weeks of heat stress. Control mice received normal saline drenches. A period of two weeks included normal saline drenching the HS group of stressed mice held at (30±1°C). A treatment period of 2 weeks using 10 mg/kg b.w. thymoquinone solution under (30±1°C) stresses was applied to the TQ group for 2 weeks

4.2. Discussion

The protective function of thymoquinone as antioxidant product cells from oxidative stress of reactive oxygen species, by effect on mitochondrial regulation and causes apoptosis, these materials prevent by enhancement of glutathione peroxidase and decrement of MDA (Hamady & Hayyawi, 2024; Hamady *et al.*, 2016). The mechanism action of thymoquinone has the modulating effect on Nrf2 signaling pathway lead to enhancement in the activity of the cell antioxidant systems (glutathione peroxidase Gpx, catalase, SOD and glutathione−s-transferase) to a decrease in the free radicals material such as hydrogen, hydroxyl, hypochloride and peroxide, lead to decrease in reactive oxygen species and lipid peroxidation and result protect cell from oxidative stress and apoptosis (Thangamany *et al.*, 2025; Chandimali *et al.*, 2025; Sadiq, 2023, Martemucci *et al.*, 2022). Thymoquinone decrease serum level of LDL while increasing in the serum levels of HDL and VLDL, these mechanism modulating of estrogen synthesis to initial estrus cycle and showed clinical sign of estrus period stages (Ramineedu *et al.*, 2024; Manoharan *et al.*, 2024; Ibrahim *et al.*, 2024; Sadeghzadeh *et al.*, 2023). As well as role of thymoquinone in cellular molecular pathway through activation of cyclooxygenase-2, related factors-2 (Nrf2), phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT), nuclear factor kappa-light-chain-enhancer of activated ß (NF-Kß) and release of cytokines all these mechanism refer to an anti-inflammatory properties (Behairy *et al.*, 2024)

Environmental stress specially in summer high increased in temperature of IRAQ climate 50°C, these due to heat stress effects on reproductive and production function in mammals, the experimental design refer to therapeutic effects of thymoquinine on heat stress through enhancement of heat shock protein as antioxidant properties by reduction free radicals that cause damage to the cell system pathway through effect on protein, enzyme synthesis and lipid peroxidation due to DNA damage (Omidi *et al.*, 2024). The mechanism action of heat shock protein HSP70 through inhibit SAPK/JNK activation that cause cell apoptosis and prevent apoptotic cell death, and activation with stabilization testis specific serine/ threonine kinase, these family responsible for fertility by enhancement of morphological change differentiation of spermatid to spermatozoa lead to enhancement of spermatogenesis (Nelson *et al.*, 2022). Thymoquinone has ability to reduce effectivity of environmental climate stress through enhancement of HSP70 has role in steroid hormonal function regulation by modulating efficacy of estrogen receptors activity, androgen receptor activity and progesterone receptors activity, these mechanism role to regulate and activate estrus cycle through enhancement of estrogen receptors and progesterone receptors that cause enhancement of estrogen efficacy lead to folliculogenesis and negative feedback mechanism on the hypothalamus to decrease serum level of FSH while increase serum LH level to cause ovulation (Hamady & Hayyawi, 2024a; Hamady & Hayyawi, 2024b).

Guide research of Physiology Department in Veterinary Medicine of Wasit University to study the role of thymoquinone on mammalian fertility and effect of environmental stress on reproductive system fertility through regulation and activation estrus cycle by therapeutic potential activity of thymoquinone on fold inhibin expression gene to embayment environment stress on adult females mice as a mammalian, the result showed role of thymoquinone to enhancement of fold inhibin expression gene, these mechanism refer to role of thymoquinone as protective function, folliculogenesis through enhanced of inhibin causes primary follicles growth, and the inhibin ß has paracrine effects in follicles development and a greater regulatory role with FSH secretion (Sukatendel *et al.*, 2025; Hamady & Hayyawi, 2024b; Alaee *et al.*, 2023). The conclusion of experiment referred to the therapeutic administration of thymoquinone as antioxidant, hormonal modulator, and potential regulation of folliculogenesis and estrus cycle activation.

5. CONCLUSION

This study highlights the potential therapeutic effects of thymoquinone on regulating folliculogenesis and activating the estrus cycle in mice subjected to stress-inducing environmental conditions. The administration of thymoquinone at 10 mg/kg b.w. significantly enhanced reproductive functions compared to the control group. The observed physiological improvements suggest thymoquinone's role in mitigating the adverse effects of heat stress. The collection and analysis of ovarian tissues revealed that thymoquinone influenced the expression of stress-related markers, such as inhibin hormone and HSP70 protein, indicating a regulatory effect on stress-induced changes. These findings underscore the potential of thymoquinone as a therapeutic agent to support reproductive health under stressful conditions. Further research is necessary to fully elucidate the mechanisms through which thymoquinone exerts its effects and to explore its applications across different stress models and species. This study contributes valuable insights into the development of interventions targeting reproductive dysfunction brought on by environmental stressors.

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