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The Effects of Thymoquinone of Black Seed Plant on Sperm Parameters, Hormones, and Oxidative Stress in Mice Poisoned by Chlorpyrifos

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Abstract

Introduction: The *Nigella sativa* plant, commonly known as black seed, contains a significant compound known as thymoquinone, which exhibits remarkable therapeutic properties. This study aimed to assess the potential therapeutic effects of thymoquinone on the adverse effects of chlorpyrifos toxicity, considering the importance of reproductive capacity and the detrimental impacts of agricultural toxins.

Materials and Methods: Various parameters were assessed in 36 NRMI male mice including testosterone and luteinizing hormone (LH) hormone secretion, sperm count and motility, as well as an antioxidant activity measured by TAC (Total Antioxidant Capacity), MDA (malondialdehyde), and SOD (Superoxide Dismutase). Both thymoquinone and chlorpyrifos were injected intraperitoneally for 14 days.

Results: In tests related to sperms, chlorpyrifos caused a severe decrease in sperm motility and number ($p<0.001$). The administration of chlorpyrifos poison caused a significant decrease in the secretion of testosterone and LH hormones ($p<0.05$). However, these adverse effects were partially reduced by thymoquinone injection together with chlorpyrifos, especially at a dose of 10 mg/kg. Based on the results of TAC, MDA and SOD antioxidant assays, chlorpyrifos poison caused a significant decrease in antioxidant capacity ($p<0.001$). In contrast, injection of thymoquinone with a dose of 10 mg/kg caused a significant improvement in the increase of antioxidant capacity in the testicular tissue ($p<0.05$).

Conclusion: According to the observed results and therapeutic effects, a dosage of 10 mg/kg thymoquinone is recommended for reduce oxidative stress-induced injury and toxin-mediated toxicity and enhance sexual traits. Overall, thymoquinone shows potential as a therapeutic agent, and further research could explore its applications in mitigating oxidative stress-related damage and improving sexual function.

Keywords: Antioxidants, Chlorpyrifos, Hormones, Spermatozoa, Thymoquinone

1. Introduction

Reproduction is a crucial biological process in human beings that holds significant importance in human existence. According to reports by the World Health Organization, there has been a steady rise in infertility statistics over the last years. The prevalence of infertility in societies is estimated to be around 13%, with a significant proportion of cases attributed to male factor infertility [1]. One of the primary causes of male infertility is testicular tissue damage, which can lead to insufficient production of healthy sperm. Hormonal imbalances are a significant contributing factor in the disruption of the process of spermatogenesis. The process of spermatogenesis is subject to damage to testicular tissue and germ cells, resulting in a reduction of healthy sperm count, which can be attributed to various factors, including oxidative stress. The vulnerability of testicular tissue and germ cells makes them susceptible to oxidative stress's detrimental consequences [2]. The use of this plant dates back to more than 3000 years ago. This medicinal annual herbaceous plant thrives in Asia, Europe, and Africa. Additionally, the traditional Iranian medicine holds a belief that it possesses the potential to treat all diseases except for death and old age [3]. This plant is a rich source of phytochemicals. Thymoquinone, thymoquinone, thymohydroquinone, and thymol substances were found in the volatile oil analysis of this plant using the liquid chromatography method. The chemical thymoquinone is this plant's most well-studied and promising therapeutic component. The molecular formula for thymoquinone is $C_{10}H1_2O_2$, making up 30-48% of the plant's primary components. Numerous studies have verified this substance's anti-cancer, antioxidant, and anti-inflammatory properties. This substance exhibits hepatoprotective properties by enhancing the activity of hepatic metabolic enzymes and acting as a non-enzymatic inhibitor of lipid peroxidation. Additionally, it mitigates the deleterious effects of oxidative stress on tissues. The presence of quinine in the molecular structure of thymoquinone, which has a significant oxidation-reduction ability, is one of two properties that contribute to the compound's strong antioxidant action. Due to the lack of limiting and inhibiting barriers, the thymoquinone compound may easily access various cell sections, eliminating and neutralizing free radicals more efficiently [4,5]. Achieving higher agricultural yields has become an area of interest due to global population growth and the need to increase food production. The use of agricultural pesticides has gradually been used for this purpose. Chlorpyrifos has been widely used as a pesticide in modern times. This toxic substance ranks among Iran's top ten most frequently utilized poisons and holds significant importance. Regrettably, the World Health Organization has classified it within the category of three hazardous toxins [6]. The poison's mechanism of action involves the inhibition of the acetylcholinesterase enzyme, resulting in detrimental effects on the respiratory system of the human body. This may manifest as respiratory disorders and, in severe cases, the fatality of the affected individual. This particular pesticide's primary mode of action involves the phosphorylation of the serine hydroxyl group present in the acetylcholinesterase enzyme. Furthermore, through the induction of oxidative stress, this toxic substance results in numerous instances of tissue damage within the human body, including those affecting reproductive tissues. The testicular tissue is susceptible to oxidative stress owing to the significant proliferation of primary germ cells and the production of generative sperms [7]. Considering the increasing pollution of the environment by chemical toxins, of which chlorpyrifos is one of the most widely used, and the sensitivity of reproductive cells to these toxins and oxidative stress, In this experimental study the effect of thymoquinone was investigated on sperm factors, sex hormones And the antioxidant capacity of testis tissue (poisoned with chlorpyrifos) in male mice.

2. Materials and Methods

This experimental research was conducted in the laboratory of Alzahra University and Islamic Azad University-Tehran Science and Research in 2023. To achieve the goals of this study, three sets of tests were conducted: evaluations of sperm count and motility, measurements of testosterone and LH hormone secretion, and assessments of TAC (Total Antioxidant Capacity), MDA (malondialdehyde assay), and SOD (Superoxide Dismutase) in testicular tissue. In this study, a total of 36 adult male NMRI mice (Royan-Iran) with a weight range of 20-25 grams were utilized. Following acclimation to the animal housing facility, the mice were randomly grouped into six cohorts. Subsequently, they administered thymoquinone and chlorpyrifos toxins via intraperitoneal injection daily for 14 days under standard conditions [3]. The study consisted of a control group and five experimental groups. The experimental groups were divided into two groups receiving thymoquinone alone at doses of 10 and 5 mg/kg, two groups receiving a combination of thymoquinone at a dose of 12 mg/kg and chlorpyrifos poison at a dose of 12 mg/kg, and one group receiving chlorpyrifos poison alone at a dose of 12 mg/kg. To administer thymoquinone, the powder (Sigma-USA) was dissolved in a solution of DSMO and normal saline, with a volume ratio 1:3 [8,9].

Evaluations of sperm count and motility

Following 14 days and subsequent anesthesia of the animals (with a mixture of ketamine 100 mg/ml, xylazine 20 mg/ml and acepromazine 5 mg/ml), the epididymal segment of the left testis was excised and subsequently homogenized in 5 ml of normal saline solution to yield a sperm suspension. Then, using a Neubauer slide and light microscope (400× magnification), normal epididymal spermatozoa were counted, and the approximate number of total spermatozoa, along with the percentage of sperm motility, was calculated [2].

Measurements of testosterone and LH hormone secretion

Blood was collected via the portal vein of the rats following the incision of their abdominal cavities. Following the centrifugation and subsequent separation of blood serum, an ELISA assay was conducted to quantify the levels of testosterone and LH hormones. The samples were prepared to utilize commercial hormone kits. Finally, the amount of the desired hormones was determined with an ELISA reader and spectrometry at a wavelength of 450 nm by comparing the optical density of the samples to that of a standard group [10].

Assessments of TAC, MDA, and SOD

TAC, MDA, and SOD were utilized to evaluate the antioxidant impact in testicular tissue. Following the lysis of a portion of testicular tissue, the test procedures for three antioxidant tests (Novin-navand Salamat, Iran) were performed. The determination of TAC of biomolecules was carried out using the ferric reducing antioxidant power (FRAP) method, which involves measuring reducing power through transferring single electrons from divalent iron. The alteration in color resulting from the chemical reaction was evaluated and computed at a specific wavelength of 593 nm [11]. Malondialdehyde is a highly reactive compound generated due to peroxidation reactions of unsaturated fatty acids. Through the process of measuring this specific complex, it is possible to calculate the quantity of lipid peroxidation. The quantification of lipid peroxidation in the MDA assay can be achieved through spectrometry of the resultant solution at a wavelength of 550 nm [12]. The desired quantity of SOD enzyme was obtained through spectrometry analysis of the final solution at a wavelength of 405 nm [13].

Statistical analysis

The statistical analysis was carried out by the SPSS software. The difference between the experimental and control groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a statistical significance of 0.05 [3].

3. Results

Evaluations of sperm count and motility

The obtained results demonstrated that thymoquinone injection significantly increased the number of sperms in the epididymal area of the testicle. Specifically, a group treated with thymoquinone at a dose of 10 mg/kg showed a statistically significant increase in sperm count ($p<0.05$) compared with the control group. Also, chlorpyrifos administration led to a significant reduction in sperm count ($p<0.001$) when compared with the normal group. The administration of thymoquinone resulted in a reduction of the adverse effects. Particularly, an experimental group receiving 10 mg/kg of thymoquinone dosage exhibited no significant difference ($p<0.05$) compared to the control group when exposed to chlorpyrifos (Figure 1). The study demonstrated a beneficial effect of thymoquinone on sperm motility. An experimental group receiving a dosage of 10 mg/kg of thymoquinone showed a statistically significant increase in sperm motility ($p<0.01$). Conversely, the administration of chlorpyrifos via injection resulted in a statistically significant decrease in sperm motility ($p<0.001$). However, the adverse effect was partially alleviated by the administration of thymoquinone at a dosage of 10 mg/kg ($p<0.01$), as illustrated in Figure 1.

Measurements of testosterone and LH hormone secretion

Our study found that thymoquinone impacted the secretion of testosterone hormone, while chlorpyrifos poison resulted in a significant decrease in hormone secretion ($p<0.01$). The administration of thymoquinone via injection was found to ($p<0.05$) mitigate the adverse impact of the toxin at both dosage levels between the two groups (Figure. 2). The impact of thymoquinone on LH secretion was observed, revealing a rise in LH secretion after thymoquinone injection. However, such an increase was not statistically significant ($p<0.05$). The administration of chlorpyrifos solely to mice resulted in a significant reduction in the secretion of LH hormone ($p<0.001$). The administration of thymoquinone in conjunction with chlorpyrifos poison resulted in a reduction of its adverse effects. The statistical analysis indicated that an experimental group treated with thymoquinone at 10 mg/kg dose did not exhibit a significant difference compared to the control group ($p<0.05$), as illustrated in Figure. 2.

Assessments of TAC, MDA, and SOD

According to the obtained results, thymoquinone in a dosage of 10 mg/kg improved the antioxidant factors in testicular tissue. The TAC antioxidant test revealed that thymoquinone alone increased the antioxidant capacity of testicular tissue. However, this effect was statistically significant ($p<0.01$) only in the group given receiving a dosage of 10 mg/kg of thymoquinone. In contrast, the antioxidant capacity significantly decreased in the chlorpyrifos-treated group, and this drop was statistically significant at the level ($p<0.001$). The co-injection of thymoquinone and chlorpyrifos increased antioxidant capacity, but only 10 mg/kg of thymoquinone caused a significant ($p<0.01$) change in antioxidant capacity (Figure. 3).

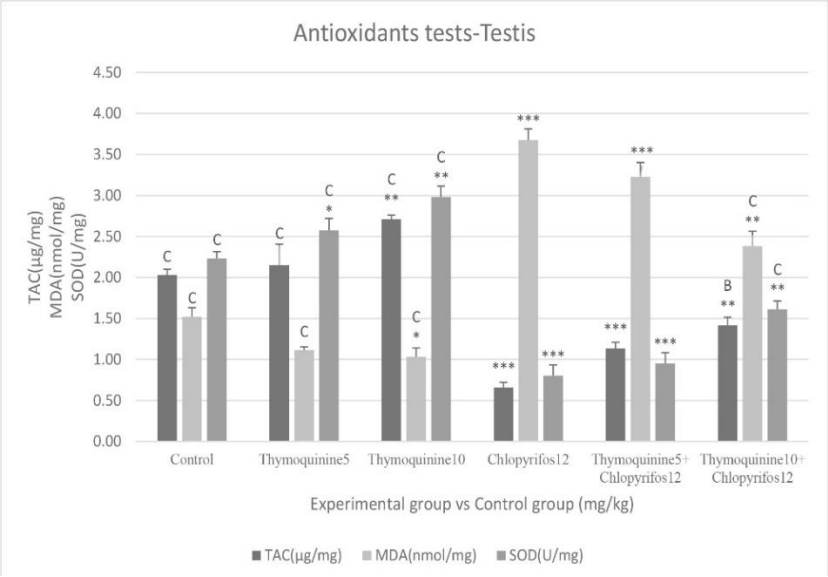

Figure. 1. The effects of thymoquinone on sperm parameters in the epididymis (sperm count and sperm motility)
Thymoquinone injection significantly increased the number and motility of sperms in the epididymal area of the testicle.
*: significant difference with the control group ($p<0.05$), **: significant difference with the control group ($p<0.01$), ***: significant difference with the control group ($p<0.001$)
B: significant difference with the chlopyrifos 12 (mg/kg) group ($p<0.01$), C: significant difference with the chlopyrifos 12 (mg/kg) group ($p<0.001$)

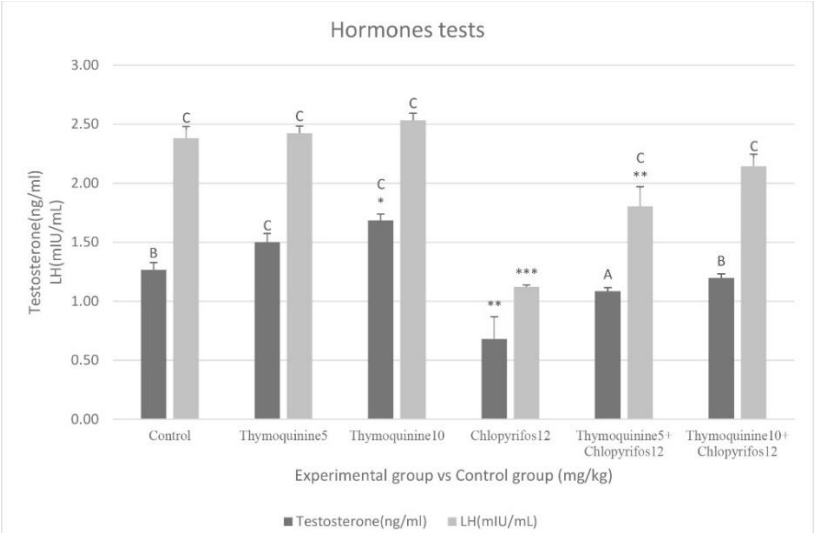

Figure 2. The effects of thymoquinone on the secretion of two hormones, testosterone and LH
Thymoquinone impacted the secretion of testosterone hormone, while chlorpyrifos poison resulted in a significant decrease in hormone secretion ($p<0.01$).

*: significant difference with the control group (p<0.05), **: significant difference with the control group (p<0.01), ***: significant difference with the control group (p<0.001)
A: significant difference with the chlopyrifos 12 (mg/kg) group (p<0.05), B: significant difference with the chlopyrifos 12 (mg/kg) group (p<0.01), C: significant difference with the chlopyrifos 12 (mg/kg) group (p<0.001)

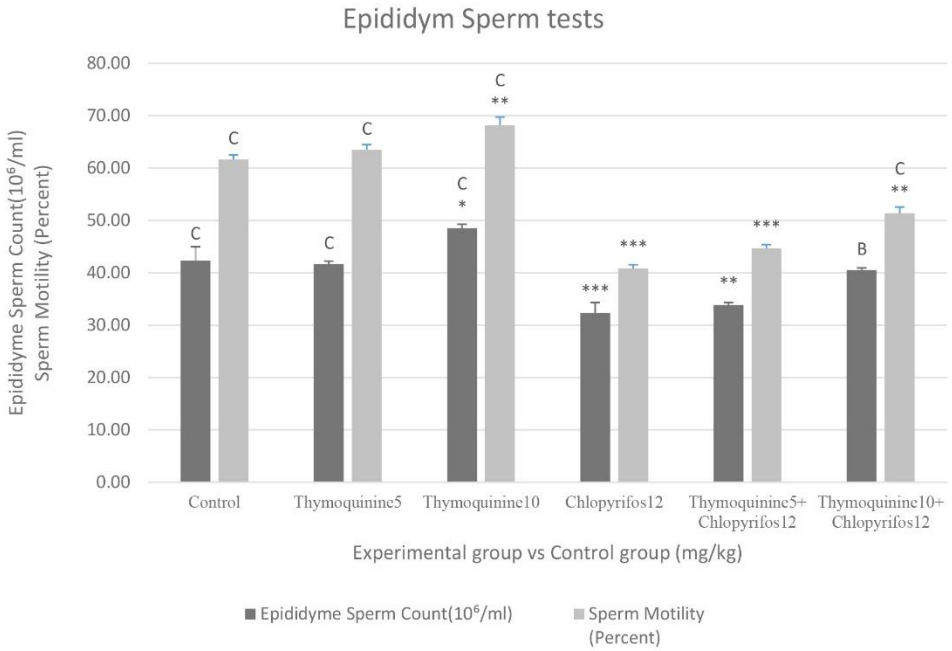

Figure. 3. Antioxidant effects of thymoquinone in testicular tissue in three TAC, MDA, SOD tests
The TAC antioxidant test revealed that thymoquinone alone increased the antioxidant capacity of testicular tissue. However, this effect was only statistically significant (p<0.01) in the group given 10 mg/kg of thymoquinone.
*: significant difference with the control group (p<0.05), **: significant difference with the control group (p<0.01), ***: significant difference with the control group (p<0.001)
B: significant difference with the chlopyrifos 12 (mg/kg) group (p<0.01), C: significant difference with the chlopyrifos 12 (mg/kg) group (p<0.001)

The analysis of the MDA antioxidant test results indicated a significant reduction in the quantity of MDA upon the administration of thymoquinone. The observed decrease was particularly significant in the group that received a 10 mg/kg dose of thymoquinone (p<0.05). The chlorpyrifos administration resulted in a significant increase in malondialdehyde (p<0.001). However, the injection of two doses of thymoquinone led to a decrease in the level of MDA. Specifically, in an experimental group receiving a dosage of 10 mg/kg of thymoquinone, a significant (p<0.01) decrease was observed (Figure. 3). Upon analysis of the SOD antioxidant test results, it was observed that the administration of thymoquinone at 5 and 10 mg/kg at dosages led to a significant rise in the quantity of this compound. The statistical significance of such an increase was observed at two statistics levels, namely p<0.05 and p<0.01. The administration of chlorpyrifos resulted in a significant reduction in the levels of SOD (p<0.001). However, receiving thymoquinone at a dose of 5 mg/kg resulted in a numerical increase, but this difference was not statistically confirmed. The amount of this molecule increased when the injection dose of thymoquinone was increased to 10 mg/kg, and this variation was statistically validated (p<0.01) (Figure. 3).

4. Discussion

The importance of the proper functioning of the male sexual organs in the ability to fertilize is well recognized. In addition, due to the increasing use of poisons, especially poisons such as chlorpyrifos with numerous destructive effects on the human body, there is a high probability of harming the reproductive organs. The therapeutic effects of the black seed plant, especially its most important substance thymoquinone, have also been observed in humans. In the current experimental research, the therapeutic effects of thymoquinone combination, the active substance of black seed plant, on the negative effects of chlorpyrifos poison on male reproduction were evaluated. The obtained results confirmed the stated aim.

Chlorpyrifos toxicity reduced sperm count and motility ($p<0.001$), but thymoquinone intraperitoneal injection, especially at 10 mg/kg dose, mitigated these harmful effects. In 2021, a study by Gur found that thymoquinone restored sperm production in damaged testes and varicocele [14]. Separate studies by Atta and Salahshoor confirmed thymoquinone's positive effects on rats with diabetes, severe obesity, and morphine treatment [15,16]. This study's findings align with previous research, showing thymoquinone's beneficial impact on spermatogenesis impaired by chlorpyrifos toxicity. Sperm cells are highly sensitive to chemical toxins, but thymoquinone may improve factors like sperm count and motility by counteracting these deleterious effects.

Chlorpyrifos significantly decreased testosterone, and LH hormone secretion ($p<0.01$), but thymoquinone at 10 mg/kg dose showed no significant difference ($p<0.05$) compared to the control group. Two studies have demonstrated the therapeutic properties of thymoquinone on sex hormone secretion in rats exposed to nicotine and lead poisoning [17,18]. In 2020 and 2022, distinct research showed the efficacy of thymoquinone in increasing the secretion of testosterone and other sex hormones in rats damaged by doxorubicin treatment and cyclophosphamide poisoning [19,20]. The present research aimed to evaluate thymoquinone's therapeutic and ameliorative properties in the context of chlorpyrifos toxicity. According to the findings, administering sex hormones testosterone and LH yielded favorable outcomes compared to experimental groups subjected to chlorpyrifos. The therapeutic effects are attributed to regulating chlorpyrifos-induced toxicity on Leydig and pituitary cells, enhancing the hypothalamic axis, pituitary gland, and testicular function.

According to TAC, MDA, and SOD results, the antioxidant capacity in the group given chlorpyrifos poison decreased ($p<0.001$). Thymoquinone, a natural compound, has shown promising antioxidant properties in various studies. In a study by Attari, thymoquinone was found to alleviate oxidative stress caused by D-galactose in mice [8]. Similarly, a study by Alaee demonstrated the antioxidant benefits of thymoquinone in improving reproductive parameters in mice with polycystic syndrome [21]. In another research by Altindağ, thymoquinone was shown to enhance sexual characteristics and act as an antioxidant in mice severely damaged by paclitaxel and diclofenac [22]. These findings are in line with previous research, highlighting the consistent antioxidant properties of thymoquinone.

Due to the limitations of the animal model study (in vivo), it is recommended to investigate the effects of thymoquinone on different tissue cell culture models (in vitro).

5. Conclusion

The present study further investigated thymoquinone's antioxidant effects using three distinct assays. Analysis of sperm factors and sex hormone secretion revealed that thymoquinone's antioxidant mechanisms likely contribute to its beneficial effects. Specifically, it protects Leydig cells responsible for testosterone secretion, mitigating the impact of chlorpyrifos toxicity, primarily in the testicles [18]. The antioxidant properties of thymoquinone are crucial due to the vulnerability of seminiferous tubules and sperm cells to free radicals during various stages of growth and development. The empirical findings suggest that a thymoquinone dosage of 10 mg/kg may effectively reduce oxidative stress-induced injury and toxin-mediated toxicity and enhance sexual traits. Overall, thymoquinone shows potential as a therapeutic agent, and further research could explore its applications in mitigating oxidative stress-related damage and improving sexual function.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Alzahra University (Ethics code: IR.AlZAHRA.REC.1401.104), and all experiments were conducted according to the ethical guidelines of this committee.

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Author's contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declare that there are no conflicts of interest.

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