Therapeutic Effect of Thymoquinone against Methotrexate-Induced Damage on Sperm Parameters in Mice

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ABSTRACT

Background: Methotrexate drug is commonly used to treat cancer and caused reproductive damage. Thymoquinone, as a natural component of herbs has many health benefits shown in researches. The present study amid to investigate probable therapeutic effect of Thymoquinone against Methotrexate-induced damage on sperm parameters in mice.

Material and methods: In this experimental study, 30 male mice (25-30 g) were divided into five groups of six in each. The mice were received normal saline (control group), Methotrexate (20 mg/kg), Methotrexate (20 mg/kg) + Thymoquinone (2, 10 and 20 mg/kg) by intraperitoneal injection. On the day after the last injection, the sperm parameters including motility, viability, and count of sperms were assessed. Data analysis was performed using one-way ANOVA followed by Turkey test.

Results: Methotrexate alone leaded to a significant reduction in sperm parameters compared to the control group (P=0.00). In groups treated with Methotrexate and Thymoquinone, sperm parameters (motility, viability, count sperm) did not showed any significant differences with control group (P=0.00).

Conclusion: Thymoquinone, as a potent antioxidant, could compensate for the toxicity induced by Methotrexate. These medical trend may be useful for diminish the side effects of Methotrexate on male reproductive system.

Keyword: Thymoquinone, Methotrexate, Sperm, Mice

INTRODUCTION

Methotrexate (MTX) as a mild immunosuppressant drug, exhibiting anti-inflammatory activity, has been represented for the treatment of some cancers [1]. However, studies in animals have shown degeneration in cellular component of seminiferous tubules affecting spermatogenesis and in men, some reports of oligospernia outbreak in psoriasis patients, both following undertaking MTX [2,3]. These harmful side effects of MTX on male reproductive system, as a part of its adverse effects, have limited the application of the drug and researches are conducted to overcome this limitation by some trends like co-administration with natural products.

Plants and their derivatives play a key role in world health and thirty percent of all modern drugs are developed from these natural resources [4]. In addition, Plants have a long folklore of use in aiding fertility, including fertility-enhancing properties and aphrodisiacal qualities [5,6].

Nigella sativa L. belongs to the botanical family of ranunculaceae [7]. It has been known as black seed and its seeds are frequently used in folk medicine in Middle East and some Asian countries for the promotion of good health and treatment of many ailments [8,9]. Nigella Sativa seed contains a complex
mixture of more than 100 constituents. Most of the therapeutic properties of *Nigella sativa* are due to the presence of a polyphenol compound named Thymoquinone (TQ) which is the major ingredient of *Nigella sativa* oil (28-57%) [10-12].

The pharmacological investigations confirmed antioxidant activity of Thymoquinone. Antioxidant property of Thymoquinone molecule which easy accesses to sub-cellular compartments facilitating the ROS scavenging [13,14]. Thymoquinone was also shown to inhibit nonenzymatic lipid peroxidation [15]. Previous data suggest that the seeds oil and Thymoquinone exhibited sperm protective effect against testes damage. Therefore, regard to value of plant used in traditional medicine for drug discovery of fertility-enhancing, this study was conducted to examine the effect of Thymoquinone against Methotrexate-Induced damage on sperm parameters in Mice.

**MATERIAL AND METHODS**

**Animals**

Thirty male Balb/c mice with weight of 25-30 g were used. Animals were kept in the temperature of 22 ± 2°C under controlled environmental conditions, 12 h light-dark cycles and fed standard pellet chow and water ad libitum. All experiments procedures were conducted in accord with the principles for the care and use of laboratory animals in research and approved by ethics committee at our university.

**Experiment protocol**

The animals were divided randomly into following 5 groups (n=6): i. Control group receiving dimethyl sulfoxide (DMSO, 1:1000) in normal saline, ii. Experimental group (E1) receiving only an intraperitoneal single dose injection of MTX (20 mg/kg; Sigma Aldrich, USA), iii. Experimental groups (E2-E4) receiving an intraperitoneal injection of MTX (20 mg/kg) plus TQ (Sigma Aldrich, USA) in different concentrations of 2 mg/kg (E2), 10 mg/kg (E3), and 20 mg/kg (E4). Experimental groups were treated over period of 4 consecutive days [16].

**Analysis of sperm parameter**

The left cauda epididymis was separated and chopped in DMEM/F12 containing 5% FBS which had been balanced in the incubator previously; it was then placed in incubator with the temperature of 37°C and 5% CO₂. To count the sperms, the solution was transferred into each chamber of Neubauer hemocytometer and sperm heads was manually counted under a microscope. Sperm count was performed according to WHO guidelines and data were expressed as the number of sperm/ml. The sperm motility was divided into four levels (48): (0): without motility, (I): minor *in situ* motility. (II): circumferential motility and (III): progressive motility. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each animal. For the assessment of viability we used from eosin y staining that discriminate life sperm from dead sperm by staining cytoplasm of cell. Counted at least 200 sperm for each animal and discriminate live sperm that was not stained and dead sperm that was red [17].

**Statistical analysis**

Results were analyzed using spss19 (SPSS Inc., Chicago, Illinois) and expressed as means ± SE. Statistical significant different was determined by one way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison. Probability values (P) less than 0.05 were considered to be statistically significant.

**RESULT**

The results of the sperm viability showed a significant increase in the alive sperm in E3 and E4 groups as compared to that of the MTX group (Figure 1a). Sperm motility and percentage of progressively motile sperm in experimental groups in comparison with MTX group had significant increase (Figure 1b). According to statistical analysis count of sperm in second (MTX+2 mg/kg of TQ), third (MTX+10 mg/kg of TQ) and fourth (MTX+20 mg/kg of TQ) experimental group in comparison with MTX group no significant increase (Figure 1c).
The results of the sperm viability showed a significant increase in the alive sperm in E3 and E4 groups as compared to that of the MTX group.

Sperm motility and percentage of progressively motile sperm in experimental groups in comparison with MTX group had significant increase.

The effect of toxic dose of MTX (20 mg/kg) and different doses of TQ on a: viability sperm b: total motility of sperms, c: sperm count, the groups (X axis) are Control: not treated, E1: MTX 20 mg/kg, E2: MTX+ TQ (2.5 mg/kg), E3: MTX + TQ (10 mg/kg), E4: MTX + TQ (20 mg/kg).

*P<0.05 compared to the control group. *** P<0.000 compared to the control group and α P<0.05 compared to the MTX group, β P<0.01 compared to the MTX group.

TMX=Methotrexate, TQ=Thymoquinone. According to statistical analyses count of sperm in second (MTX+2 mg/kg of TQ), third (MTX+10 mg/kg of TQ) and fourth (MTX+20 mg/kg of TQ) experimental group in comparisons with MTX group no significant increase.

DISCUSSION

In the present study, the Therapeutic effect of TQ against Methotrexate -induced cytotoxicity in mice was evaluated. The results showed a significant decline in the sperm parameters of animals treated with Methotrexate. Methotrexate is a well-known anti-cancer agent used for the treatment of malignant and non-malignant conditions. In recent years, large number of reports has been published on potential gonadal damage following drug toxicity by anti-cancer drugs both on cellular and molecular aspects during spermatogenesis. Gonad is an important component of reproductive system, associated with series of cellular interaction, differentiation to form mature germ cells through process of spermatogenesis. Any insult at this stage on gonads may impair fertility [3-17]. Previous study also indicated that low dose of MTX affects cellular contents, diameter of seminiferous tubules and interstitial space of testis during spermatogenesis.

Infertility is a complex disorder with significant medical, psychosocial and economic aspects. About 25% of couples do not achieve pregnancy within 1 year, 15% of whom seek medical treatment for infertility and less than 5% remain unwillingly childless. Infertility affects both men and women. Male causes for infertility are found in 50% of involuntarily childless couples [18].

A wide majority of medicinal plants possess pharmacological principles, which has rendered them useful as curatives for numerous ailments. According to the World Health Organization (WHO) reports, 70-80% of the world population confide in traditional medicine for primary health care [19,20].

TQ is the major active component derived from Nigella sativa and reported that many of the pharmacodynamics effects. Nigella sativa are due to TQ [21,22]. TQ treatment has protective effects on testicular parameters. The results of present study are in correlating with previous studies and confirmed that the testicular favoring results due to TQ content in Nigella sativa seed. So the Thymoquinone may be used for increasing testicular
activity. Several case reports and series have documented reversible sterility in men using Methotrexate [23,24]. They reported a decrease in sperm count or quality with use of the agent. When the medication was discontinued, the sperm returned to normal levels and quality. Replicate DNA due to inhibition of an essential enzyme dihydrofolate reductase required for normal DNA synthesis. Therefore, it can be concluded that these qualitative and quantitative changes in male gonads may alter the reproductive performance of animals, if not reversible in nature. However, further study is required at ultra-structural and molecular level to explore the mechanism of action of methotrexate. De Luca et al. also reported minimal to no suppression of spermatogenesis with methotrexate therapy [25]. Oxidative stress is harmful to sperm function and a significant factor in the etiology of male infertility [26]. In addition; oxidative stress impairs male fertility by changing the cell function like sperm motility, increase in DNA damage by induction of gene mutations, DNA denaturation, base pair oxidation and DNA fragmentation [27]. Many factors lead to the sperm abnormality especially sperm without head. Lipid peroxidation and accumulation of free radicals cause morphological damage of sperm [28].

CONCLUSION

In conclusion, present study demonstrated that the therapeutic effect of Thymoquinone against Methotrexate-Induced damage on sperm parameters in Mice. Thymoquinone as antioxidants may improve fertility by means of increasing the sperm parameters.

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