Original Article

Effect of Thymoquinone on Reproductive Parameter in Morphine-treated Male Mice

Mohammad Reza Salahshoor, Mojdeh Haghjoo, Shiva Roshankhah, Fatemeh Makalani, Cyrus Jalili

From the Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Background: Thymoquinone as the main active component of *Nigella sativa* might have a various pharmacological effects such as antiapoptotic and antioxidant. Morphine is commonly used for the treatment of severe pain that can increase the generation of free radicals and affects the spermatogenesis. This study was designed to evaluate protective effects of thymoquinone against morphine-induced damages, sperm viability, count, motility, morphology and testis histology, and nitric oxide and testosterone hormone of the mice. **Materials and Methods:** In this experimental study, we divided 48 mice into eight groups (n = 6); various doses of thymoquinone (2, 10, and 20 mg/kg) and morphine (20 mg/kg) plus thymoquinone (2, 10, and 20 mg/kg) were administered intraperitoneally to 48 male mice for 30 consequent days. Male reproductive parameters including testis weight, testosterone hormone, serum nitric oxide, germinal thickness, sperm morphology, count, viability, and motility were analyzed and compared. **Results:** The results indicated that morphine administration significantly decreased germinal thickness, testis weight, testosterone level, viability, morphology, count, and motility of sperm and increased nitric oxide as compared to saline group ($P < 0.05$). However, increasing the dose of thymoquinone in the thymoquinone and thymoquinone plus morphine groups significantly decreases nitric oxide level ($P < 0.05$) while significantly boosted motility, morphology, count, viability of sperm cells, germinal thickness, and testosterone hormone in all groups as compared to morphine group ($P < 0.05$). **Conclusion:** It seems that thymoquinone administration could increase the quality some of spermatozoa and improves morphine-induced adverse effects on reproductive parameters in male mice

Keywords: *Morphine, reproductive, thymoquinone*

Introduction

Infertility is one of the major problems among young couples, and one out of every six couples is suffering from infertility. Factors such as genetics, job, and environment cause infertility.[1] Drugs change the function of sexual organs, the most well-known and oldest type of which is opium. Opiates affect pituitary system, sex organs, and sexual functions.[2] Morphine is the main component of opium latex, and long-term consumption of opium or morphine can be followed by reduced testosterone and reduced number of sperms consequently.[3] Regular use of morphine, especially in patients using morphine sulfate vials medically for relieving pain, can decrease the number of sperms and cause sperm deformity and dysfunction in men.[4] Like other opiates, morphine exerts its effect through opioid receptors. These receptors are dispersed in the limbic system, amygdala, hippocampus, thalamus, and hypothalamus and exist in testes too.[5] Opiates affect pituitary system, sexual organs, and sexual abilities. In the study of Ghowsi *et al*., daily consumption of methadone for 5–10 days was found to have a significantly negative effect on the weight of subsidiary sexual organs in the laboratory mice.[6] Hypothalamus, as the control center of hypothalamic–pituitary–gonadal axis, receives its nerve impulses from many brain centers such as amygdala, thalamus, pons, and cortex, which are alternatively responsible for the secretion of pituitary system and gonads.[7] Gonadotropin-releasing hormone (GnRH), secreted from hypothalamus, stimulates the secretion of GnRH and luteinizing hormone (LH) from the anterior pituitary gland; both in turn triggering testis performance.[8] LH affects Leydig cells and causes the production of testis steroids.

How to cite this article: Salahshoor MR, Haghjoo M, Roshankhah S, Makalani F, Jalili C. Effect of Thymoquinone on Reproductive Parameter in Morphine-treated Male Mice. Adv Biomed Res 2018;7:18.

Received: April, 2017. Accepted: June, 2017.

Address for correspondence:
Dr. Cyrus Jalili, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail: cjalili@yahoo.com

Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_69_17

Quick Response Code:

On the other hand, follicle-stimulating hormone (FSH) is the main stimulant of seminiferous tubules of the testis.[9] Seminiferous tubules are responsible for exocrine functioning and sperm production. Daniell *et al*. showed the inhibiting effect of opiates on GnRH production.[10] Furthermore, morphine can increase the production of free radicals by activating lipid peroxidation, thereby blocking antioxidant enzymes and causing the formation of free radicals reactive oxygen species (ROS).[11] These free radicals cause cell membrane impairment and DNA segmentation.[12] Given the side effects of the conventional drugs, the use of plant and natural products as traditional treatment has increased the number of diseases in recent decades.[13] Many of these compounds have preventive effects and can be used to inhibit special illnesses. The use of medicinal plants in traditional medicine is of great significance, and they have been used long before for the treatment of a number of diseases.[14] One of these plants is *Nigella sativa* Linn, an annual plant of *Ranunculaceae* family, which is the natural flora of Southern Europe, North Africa, and Southwestern Asia. The seeds of this plant contain oil, protein, alkaloids such as ni–glycine and ni–glydine, and quinones such as thymoquinone, saponin, and volatile essence.[15] The pharmacologic and biologic effects of *N. sativa* and thymoquinone, as the components of *N. sativa*, include antioxidant, anti-ischemic, anti-inflammation, analgesic, antiepileptic, and anticough effects.[16] Studies carried out on *N. sativa* have indicated that it has antibacterial, anticonvulsant, and antioxidant properties, reduces blood glucose, and collects the free radicals.[17] Considering the toxic effects of morphine and properties of thymoquinone and that no study has ever evaluated the effects of thymoquinone on the morphine-induced impairments, the current study was conducted to assess the effect of thymoquinone on the morphine-induced impairments in some reproductive parameters of male animal models.

Materials and Methods

Animals

In this experimental study, 48 Balb/c male mice, weighed 27–30 g, were purchased from Tehran Pasteur Institute and kept at the animal house of Kermanshah University of Medical Sciences in special standard cages. Furthermore, before the experiment, the animals were fed normal diet and water to get used to the environment and establish physiological adaptation. In this period, the mice were kept at similar conditions, temperature of 22 ± 2°C, 12 h light and 12 h darkness, and free access to water and food. All experimentation was conducted under approval of the Ethics Committee of Kermanshah University of Medical Sciences.[2]

Chemicals

Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone; $C_{10}H_{12}O_2$) and morphine ($C_{16}H_{19}NO_3$) were obtained from Sigma Chemical Company (St. Louis, USA) and were dissolved in saline (0.9%) for administration.[16]

Experimental protocol

A total of 48 male mice were divided into eight groups (n = 6): Group 1 received morphine administered by intraperitoneally injection [Table 1]; Group 2 received thymoquinone (2 mg/kg); Group 3 received thymoquinone (10 mg/kg); and Group 4 received thymoquinone (20 mg/kg). Mice with thymoquinone as follows: once daily for 30 consecutive days, intraperitoneally injecting. Group 5 received thymoquinone (2 mg/kg) plus morphine (20 mg/kg); Group 6 received thymoquinone (10 mg/kg) plus morphine (20 mg/kg); Group 7 received thymoquinone (20 mg/kg) plus morphine (20 mg/kg); and Group 8 received 0.9% normal saline. Mice with morphine plus thymoquinone as follows: once daily for 30 consequent days, intraperitoneally injecting. The same volume of saline was administered.[3,18]

Testes weight measurement

The animals were anesthetized with chloroform, and blood samples were taken from the heart to assess hormone and nitric oxide levels. Animals were killed and sacrificed. Testes were removed and weighed on a microbalance sensitive to 0.001 mg (Precisa 125A, Switzerland) and recorded.[3]

Hormone assay

Blood serum was separated from collected blood using a centrifuge (4000 g for 10 min). The serum samples were kept in deep freezer at −18°C. The blood testosterone concentrations were measured by the ELISA (Abcam 108666, USA) method.[3]

Serum nitric oxide measurement

Nitric oxide measurement was performed by Griss assay using microplate method. In sum, sulfonamide solutions, N-(1-naphthyl) ethylenediamine dihydrochloride (NEED), and nitrite standards were prepared. To measure nitrite concentration in serum, samples de-freezing, 100 µl of the sample serum was deproteinized by zinc sulfate (6 mg zinc sulfate powder was mixed with 400 µL serum and vortexed for 1 min.) and transferred to the wells. To recover nitrate to nitrite, 100 µl chloride vanadium, 50 µl sulfonamide, and 50 µl NEED solutions were added afterward (vanadium chloride recovery [III] method). Samples' optical density (OD) was measured by ELISA reader at the wavelength of 540 nm.[16]

Table 1: Morphine administered

Treatment days	1-5	6-10	11-20
Morphine administered dose (mg/kg)	20	20	30
Frequency of injection	Once daily	Twice per day	Twice per day

Sperm count

To count the sperm cells, a small amount of prepared epididymal sperm suspensions were diluted with formaldehyde fixative (10% formalin in phosphate buffered saline). We diluted 400 μL of the sperm suspension with formaldehyde (Sigma, USA), and approximately 10 μL from the diluted solution was transferred into a Neubauer chamber using a Pasteur pipette (Thoma, Assistant, Sondheim/Rhön, Germany), and the solution was allowed to remain for 7 min. Then, the sperms at the four corners of the central square were counted.[11]

Sperm viability

Sperm viability was evaluated using eosin Y staining (5% in saline). Freshly sperm suspension (40 μL) was placed on a glass slide, mixed with 1% eosin, and observed by a light microscope (×400) after smear was allowed to air-dry on a glass slide. Live sperms remained unstained following staining. At least 250 sperms were counted from each sample in ten fields and the ratio of live sperms was verified.[19]

Sperm motility

The percentage of motile sperm was evaluated using a light microscope (Olympus Co., Tokyo, Japan) at 400× magnification. For this process, one drop of sperm suspension was placed on the chamber. Sperm motility was divided into four levels according to certain criteria; slowly progressive forward movement, rapid progressive forward movement, residual motion and those motionless were counted in several microscopic fields and percentages of motile and immotile sperm cell were acquired. Motility estimates were obtained from five different fields in each sample. The mean of the five successive estimations was used as the final motility score.[3]

Sperm morphology

The sperm morphology was evaluated by analysis of sperm smears made from left cauda epididymis. An aliquot of the sample was used for preparing the smears to evaluate the spermatozoa deformities. Papanicolaou method was used to estimate spermatozoa morphology. A total of 300 spermatozoa were analyzed on each slide (3000 cells in each group) for abnormalities of the head and tail.[19]

Germinal layer thickness

After testes preserved by formalin, the histological process including dehydrating, clearing, and embedding was carried out. The microscopic sections (5 μm) were prepared and hematoxylin and eosin staining method was used. Germinal layer thicknesses were measured by Motic camera and software (Moticam 2000, Spain). Germinal layer average diameter (μm) was determined for each testis.[19]

Statistical analysis

All data are presented as mean ± standard deviation. Statistical differences among groups were carried out one-way analysis of variance (ANOVA), followed by the LSD *post hoc* test, to determine the statistical significance between different groups using the Statistical Package for the Social Sciences software (version 16.0, SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Weight of testis

The morphine caused a significant decrease in testis weight a compared to saline group ($P < 0.05$). Thymoquinone improved testis weight in all doses administration compared with the morphine group ($P < 0.05$). Testis weight was significantly increased in mice treated with thymoquinone and thymoquinone plus morphine in all doses administration in comparison with morphine group ($P < 0.05$) [Table 2].

Testosterone hormone and nitric oxide

Morphine caused a significant decrease in the testosterone hormone as compared to the saline (control) group ($P < 0.05$). Increasing doses of thymoquinone and thymoquinone plus morphine administration showed significantly increased testosterone in all groups compared to the morphine group ($P < 0.05$). Thymoquinone prevented the damage caused by morphine on testosterone level [Figure 1]. The mean level of nitric oxide in

Table 2: Different reproductive parameters between treatment groups

Parameters	Groups								*P*
	Saline	Morphine	T 2 mg/kg	T 10 mg/kg	T 20 mg/kg	T/M 2 mg/kg	T/M 10 mg/kg	T/M 20 mg/kg	
Normal morphology (%)	78.33±1.6	43.16±2.1	71.33±2.4	77±2.01	75±1.3	53.15±1.4	56.83±1.5	57.73±1.3	<0.05
Testis weight (g)	0.10±0.002	0.08±0.004	0.11±0.005	0.11±0.012	0.11±0.09	0.10±0.002	0.10±0.014	0.098±0.016	<0.05
Sperm count (106)	2.88±0.11	0.64±0.2	2.65±0.47	2.25±0.56	1.8±0.14	1.46±0.28	1.3±0.17	1.88±0.75	<0.05
Fast motility (%)	62.33±1.04	2±0.12	78.73±0.6	99.13±0.8	51.83±1.07	11.5±0.3	11.23±0.2	5.44±0.5	<0.05
Sperm viability (%)	76.93±1.5	54.01±3.3	84.72±2.2	91.42±2.09	84.06±1.4	61.45±3.3	65.81±1.2	61.21±3.1	<0.05

Statistical differences among groups were carried out ANOVA. $P<0.05$ was taken as the level of significant, morphine group compared to the saline group, all doses of T and T/M groups compared to morphine group. Data were presented as mean±SD. T: Thymoquinone, T/M: Thymoquinone plus morphine, ANOVA: Analysis of variance, SD: Standard deviation

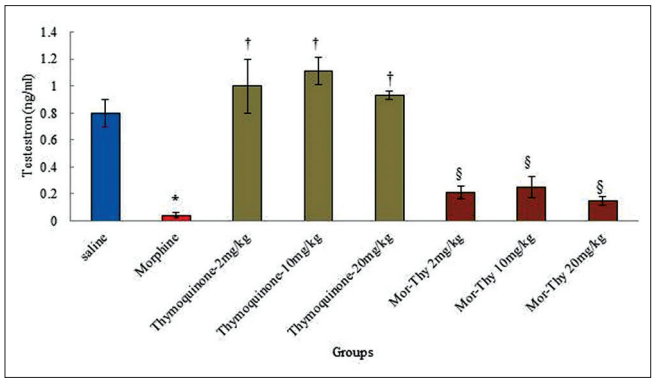

Figure 1: Effects of morphine, thymoquinone, and thymoquinone plus morphine on means of testosterone hormone levels in animals (*n* = 6 for each group). *Significant decrease in morphine group compared to saline group (*P* < 0.05). †Significant increase in all thymoquinone groups compared to morphine group (*P* < 0.05). §Significant increase in all doses thymoquinone plus morphine groups compared to morphine group (*P* < 0.05)

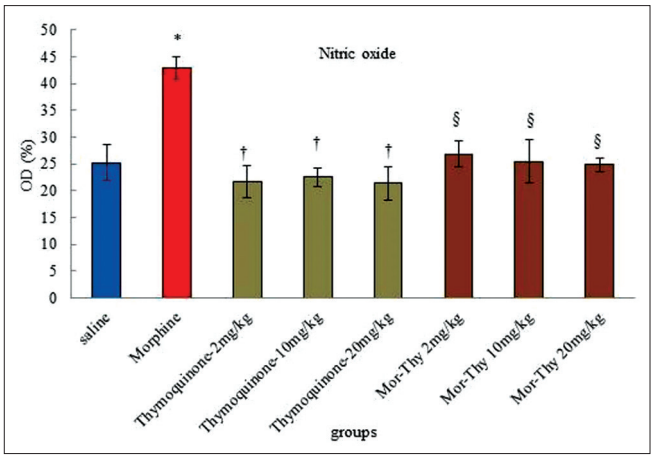

Figure 2: Correlation analysis between treatment groups (thymoquinone, morphine, and thymoquinone plus morphine) in mice and nitric oxide in the blood serum. *Significant increase in morphine group compared to saline group (*P* < 0.05). †Significant decrease in all doses thymoquinone groups compared to morphine group (*P* < 0.05). §Significant decrease in all doses thymoquinone plus morphine groups compared to morphine group (*P* < 0.05)

blood serum (OD) increased significantly in morphine group ($P < 0.05$). Thymoquinone and thymoquinone plus morphine in all groups administration cause decrease level of nitric oxide compared to morphine group ($P < 0.05$) [Figure 2].

Sperm parameters

The sperm viability, mean sperm count, normal morphology, and sperm progressive motility significantly decreased in the morphine administration group ($P < 0.05$). However, motility, count, normal morphology, and sperm viability were significantly improved in thymoquinone and thymoquinone plus morphine in all treated groups compared with the morphine group ($P < 0.05$) [Table 2].

Germinal layer thickness

Morphine administration caused a significant decrease in the germinal layer diameters ($P < 0.05$). Thymoquinone improved germinal thickness in all doses administration compared with the morphine group ($P < 0.05$). Thymoquinone plus morphine in all treated groups caused a significant increase in germinal layer diameters compared with the morphine group ($P < 0.05$) [Figures 3 and 4].

Discussion

Drug addiction is one of the problems of the current world. It seems that drug use can affect spermatogenesis and sperms by changing the sexual functions and related hormones. Medicinal plants are one of the target tissues for plant extracts in reproductive organs. The present study evaluated the protective effects of thymoquinone on the morphine-induced disorders in sperm parameters such as motility, count, viability, morphology as well as changes in the weight and structure of testis, and nitric oxide and testosterone levels in the blood serum of mice. The results of testis weight analysis in the study groups showed that morphine administration reduced testis weight. The size and weight of testis are intensely associated with the number of Sertoli cells and sperm production so that the testis size is reflective of the number of germinal cells in the testis.[20] It seems that this is due to production of free radicals and ROS by morphine and effect of these harmful factors on the death of testis sensitive cells.[3] Thymoquinone, as an antioxidant, influences testis, accessory glands, epididymis, and sperm and prevents the damage in free radicals.[21] Further, the increasing effects of thymoquinone on LH and FSH and constructive effects of these two hormones on testis structure can affect testis weight increase.[22] The study of Jalili *et al*. showed that morphine consumption significantly reduced the weight of sexual organs in the mice, and these effects were largely reduced by genistein consumption, an antioxidant, confirming the results of the present research.[3] In the current study, morphine significantly reduced sperm count, and the decreasing effects of morphine on sperm count were greatly eliminated after thymoquinone administration. Morphine reduces the number of sperms through apoptotic increase and delayed cell division cycle.[23] It seems that thymoquinone, through inhibition of cell death by antioxidant mechanism, plays a pivotal role in the preservation and integrity of mitochondria. Mitochondria play a significant role in apoptosis in oxidative stress conditions by releasing C cytochrome.[24] The findings of Rahman *et al*. showed that thymoquinone effects on stress-induced changes in reproductive system in the groups receiving thymoquinone increased sperm count and testis weight as compared to the groups under stress.[25] In addition, thymoquinone can reduce mitochondrial membrane potential, caspase-3 activity, and apoptotic cascade activity by releasing cytosolic calcium.[26] Reduction of sperm count in morphine-receiving groups can be the direct result of increased lipid peroxidation due to oxidative stress, which has been able to change the natural properties of membrane,

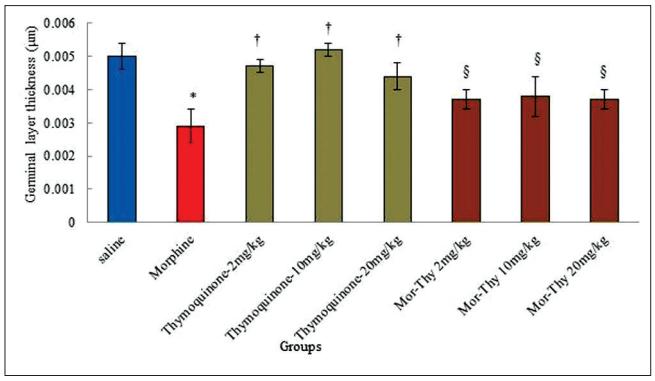

Figure 3: Correlation analysis between treatment groups (morphine, thymoquinone, and thymoquinone plus morphine) in Balb/c mice and germinal layer thickness of seminiferous tubules. *Significant decrease in morphine group compared to saline group ($P < 0.05$). †Significant increase in all doses thymoquinone groups compared to morphine group ($P < 0.05$). §Significant in all thymoquinone plus morphine doses groups compared to morphine group ($P < 0.05$)

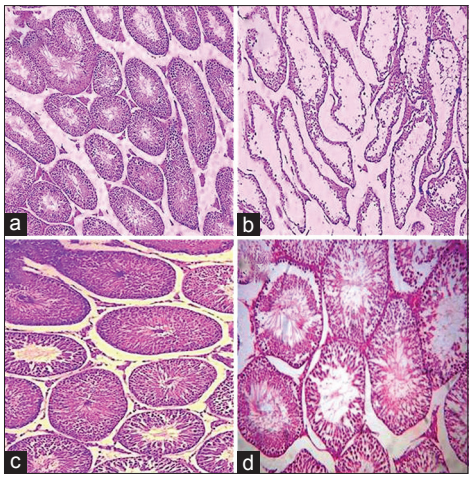

Figure 4: Effects of different concentrations of morphine, thymoquinone, and thymoquinone plus morphine on the germinal layer thickness of seminiferous tubules according to hematoxylin and eosin staining. (a) Cross-section from the testis of mice from the saline group with normal seminiferous tubules (40×), (b) morphine (20 mg/kg) (40×), (c) thymoquinone (10 mg/kg) (40×), (d) thymoquinone plus morphine (10 mg/kg) (40×)

thereby leading to the loss of sperms transferring to epididymis and present in epididymis, and antioxidants such as thymoquinone can reduce this trend.[3,16] The results of the present research revealed the effect of thymoquinone administration on increasing testosterone level after reducing it due to morphine in the study groups. Opioids such as morphine exert their effect through opioid receptors in the limbic system, thalamus, hypothalamus, and testis. Potentially existing in the pituitary gland and testicles and by bonding to opioid receptors in hypothalamus, morphine regulates the performance of gonads. Hypothalamus, as the control center of hypothalamic–pituitary–gonadal axis, receives its nerve impulses from many brain centers such as amygdala, thalamus, pons, and cortex and is alternatively responsible for pituitary and gonadal secretion.[27] Morphine use decreases LH serum level and inhibits LH-dependent spermatogenesis.[28] Since opioid receptors have also been identified in the testis tissue, morphine may inhibit spermatogenesis by directly affecting the opioid receptors of testis tissue.[29] The findings of Salahshoor *et al*. showed that morphine administration in male mice reduced testosterone level in comparison with control group, confirming the results of the present study.[11] It seems that unsaturated fatty acids in *N. sativa* oil stimulate the activity of ß-hydroxysteroid dehydrogenase; this enzyme is involved in testosterone synthesis pathway.[30] *N. sativa* extract can also lead to testosterone biosynthesis through increasing the activity of hypothalamic–pituitary–testicular axis as well as LH secretion.[31] The results of Mabrouk *et al*. indicated the increased level of testosterone hormone in the mice undergoing thymoquinone treatment, which is in line with the findings of the current study.[22] Nitric oxide can play a vital role in sperm physiology and have numerous negative effects on hypothalamic–pituitary–testicular axis.[32] In the present study, morphine exerted increasing effects on blood nitric oxide, which were remarkably reduced by administration of thymoquinone. Studies have also shown a relationship between nitric oxide and sperm acrosome and tail in the mice and human. It seems that nitric oxide can have reducing effects on sperm motility by decreasing ATP level.[33] Nitric oxide can impair sperm mitochondrial membrane, thereby releasing C chromosome, causing caspase cascade activity and stimulating apoptosis.[3] Morphine can induce increased NO production by intracellular regulation of calcium and activating calcium/calmodulin-dependent nitric oxide synthase.[34] Apparently, antioxidants are able to eliminate the free radicals. Antioxidants impair NO system (protein enzymes, substrates, and cofactors), thereby decreasing NO production.[16] The results of Jalili *et al*. showed that *Petroselinum crispum* extract, as a potent antioxidant, can reduce NO and have positive effects on sperm parameters, which is in line with the results of the present research.[19] The results of germinal layer thickness analysis in the study groups showed the significantly decreasing effects between the groups receiving morphine as compared to other groups, indicating the improvement of germinal layer thickness after thymoquinone treatment. It seems that seminiferous tubules cells in the morphine-receiving groups are quickly differentiated. Lipid peroxidation, by increasing double bonds in the cell membrane, causes the impairment and instability of cytoplasmic bridges among them, which causes their release from the tubules and reduction of germinal layer thickness of seminiferous tubules consequently.[3] Considering its different properties, especially antioxidant activity, thymoquinone can have a protective role for germ cells and can increase the germinal layer thickness by neutralizing the destructive effects of morphine.[18] The findings of Jalili *et al*. on the protective effects of utrica diocia on the internal diameter of seminiferous tubules against nicotine-induced impairments confirm the results of the present study.[35] The motility and viability of sperms are the most important parameters to

measure the ability of sperm fertilization. In the current study, thymoquinone showed protective effects on sperm motility and viability against morphine-induced damages. Sperm membranes of mammals contain a large amount of unsaturated fatty acids that are sensitive to lipid peroxidation resulting from oxidative stress, which causes the rapid loss of intracellular ATP and reduction of sperm motility and viability. Moreover, DNA damages have been observed due to oxygen free radicals and oxidative stress.[11,36] Therefore, it seems that reduced viability and motility of sperms in morphine-treated rats are due to the potential of this substance in inducing oxidative stress by lipid peroxidation of sperm membranes. In general, increased ROS in semen can induce lipid peroxidation, membrane damage, inactivation of glycolic enzymes, impairment of acrosome membrane, and DNA oxidation, thereby reducing sperm motility and viability.[3] It seems that the protective role of thymoquinone against morphine in the present study is associated with antioxidant properties and collecting ability of free radicals by this substance.[17] The results of Ping *et al*. demonstrated that *N. sativa* extract significantly increased sperm motility and viability in the groups receiving nicotine, which is in agreement with the results of the present study.[37] On the other hand, Alhimaidi *et al*. showed that using thymoquinone in the culture medium of sperms as well as in fertilization of the mice eggs in intracytoplasmic sperm injection reduced sperm progressive motility and embryo fertilization and development in the culture medium, which is in contrast with the findings of the current study.[38] The results of sperm morphology in the study groups indicated a significant reduction between the morphine-receiving groups compared with saline-receiving groups. Further, significant changes were found in terms of normality of sperms in the groups receiving thymoquinone and thymoquinone plus morphine in comparison with morphine group alone. A direct association seems to exist between the ROS level produced and percentage of sperms with abnormal morphology. The more the percentage of sperms with abnormal morphology in sperm population, the higher the ROS level produced and the more sperms are exposed to impairments induced by lipid peroxidation and oxidative stress.[39] Since a high volume of sperm cytoplasm is lost during spermatogenesis (lack of antioxidant systems), they are more sensitive to increased ROS than somatic cells. The first outcome of ROS invasion to membrane structures is occurrence of cellular peroxidation inside the cell membrane and organelles.[39] Antioxidants such as thymoquinone, as repellents of toxic materials and free radicals from the surroundings of cells, can maintain the biochemical structure of cells.[16] In addition, opioids can greatly affect the morphology, count, and motility of sperms by reducing gonadotropins and directly influencing the testis structure.[40] In the study of Daniel *et al*., intense morphologic changes of the sperms were observed in microscopic analysis of the semen of addicts, which confirms the findings of the present study.[10] Given the properties of thymoquinone in enhancing sex hormones, eliminating free radicals, and directly affecting the structure of testis, accessory glands, epididymis, and sperms,[21] it seems that this substance can have a protective role against sperm morphology alterations and other sperm parameters. In general, some studies have reported thymoquinone as a substance that increases fertility in the male gender.[41] The results of the current study showed that thymoquinone, as the effective material of *N. sativa* and an antioxidant, can have protective effects in spermatogenesis process against morphine-induced injuries, which is mainly caused by oxidative stress.

Conclusion

The present study showed that thymoquinone can significantly improve some of spermatogenesis parameters in mice treated with morphine. The results also suggest the protective potential of thymoquinone, especially antioxidant effects, against toxic effects of morphine-treated male mice. However, further research in animal models is required for better understanding for the molecular interaction between thymoquinone and morphine mechanism, leading to changes of spermatogenesis, and other pharmacokinetic parameters of thymoquinone are still incomplete.

Acknowledgments

We gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (No: 94093) for the financial support. This work was performed in partial fulfillment of the requirements for MD of Mojdeh Haghgo in Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Financial support and sponsorship

The study was financially supported by the Research Council of Kermanshah University of Medical Sciences (No: 94093).

Conflicts of interest

There are no conflicts of interest.

References

1. Gannon JR, Walsh TJ. The epidemiology of male infertility. In: Biennial Review of Infertility. Vol. 41. Urol Clin North Am 2015. p. 195-204.
2. Salahshoor MR, Khashiadeh M, Roshankhah S, Kakabaraei S, Jalili C. Protective effect of crocin on liver toxicity induced by morphine. Res Pharm Sci 2016;11:120-9.
3. Jalili C, Ahmadi S, Roshankhah S, Salahshoor M. Effect of Genistein on reproductive parameter and serum nitric oxide levels in morphine-treated mice. Int J Reprod Biomed (Yazd) 2016;14:95-102.
4. Takzare N, Samizadeh E, Shoar S, Majidi Zolbin M, Naderan M, Lashkari A, *et al.* Impacts of morphine addiction on spermatogenesis in rats. Int J Reprod Biomed (Yazd) 2016;14:303-8.
5. Vicente-Carrillo A, Álvarez-Rodríguez M, Rodríguez-Martínez H.

The mu (μ) and delta (δ) opioid receptors modulate boar sperm motility. Mol Reprod Dev 2016;83:724-34.
6. Ghowsi M, Yousofvand N. Impact of morphine dependency and detoxification by methadone on male's rat reproductive system. Iran J Reprod Med 2015;13:275-82.
7. Jin JM, Yang WX. Molecular regulation of hypothalamus-pituitary-gonads axis in males. Gene 2014;551:15-25.
8. Kim NN, Shin HS, Choi YJ, Choi CY. Kisspeptin regulates the hypothalamus-pituitary-gonad axis gene expression during sexual maturation in the cinnamon clownfish, *Amphiprion melanopus*. Comp Biochem Physiol B Biochem Mol Biol 2014;168:19-32.
9. Ciarrocca M, Capozzella A, Tomei F, Tomei G, Caciari T. Exposure to cadmium in male urban and rural workers and effects on FSH, LH and testosterone. Chemosphere 2013;90:2077-84.
10. Daniell HW. Hypogonadism in men consuming sustained-action oral opioids. J Pain 2002;3:377-84.
11. Salahshoor MR, Khazaei M, Jalili C, Keivan M. Crocin improves damage induced by nicotine on a number of reproductive parameters in male mice. Int J Fertil Steril 2016;10:71-8.
12. Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: Mechanisms, inhibition, and biological effects. Biochem Biophys Res Commun 2005;338:668-76.
13. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016;79:629-61.
14. Nejatbakhsh F, Shirbeigi L, Rahimi R, Abolhassani H. Review of local herbal compounds found in the Iranian traditional medicine known to optimise male fertility. Andrologia 2016;48:850-9.
15. Akram Khan M, Afzal M. Chemical composition of *Nigella sativa* Linn: Part 2 Recent advances. Inflammopharmacology 2016;24:67-79.
16. Jalili C, Salahshoor MR, Hoseini M, Roshankhah S, Sohrabi M, Shabanizadeh A. Protective effect of thymoquinone against morphine injuries to kidneys of mice. Iran J Kidney Dis 2017;11:142-50.
17. Paseban M. Effect of hydro-alcoholic extract of *Nigella sativa* on gastric acid and mucus secretion in cyclooxygenase inhibition condition in rats. Horiz Med Sci Persian 2014;20:109-13.
18. Sheikhbahaei F, Khazaei M, Rabzia A, Mansouri K, Ghanbari A. Protective effects of thymoquinone against methotrexate-induced germ cell apoptosis in male mice. Int J Fertil Steril 2016;9:541-7.
19. Jalili C, Salahshoor MR, Naderi T. The effect of hydroalcoholic extract of P. crispum on sperm parameters, testis tissue and serum nitric oxide levels in mice. Adv Biomed Res 2015;4:40.
20. Slegtenhorst-Eegdeman KE, de Rooij DG, Verhoef-Post M, van de Kant HJ, Bakker CE, Oostra BA, *et al*. Macroorchidism in FMR1 knockout mice is caused by increased Sertoli cell proliferation during testicular development. Endocrinology 1998;139:156-62.
21. Tawfeek FK, Kakel SJ. Effect of *Nigella sativa* oil treatment on the sex organs and sperm charactors in rats exposed to hydrogen peroxide. Mesopotamia J Agric 2006;34:1-7.
22. Mabrouk A, Ben Cheikh H. Thymoquinone supplementation ameliorates lead-induced testis function impairment in adult rats. Toxicol Ind Health 2016;32:1114-21.
23. Kaushal N, Bansal MP. Dietary selenium variation-induced oxidative stress modulates CDC2/cyclin B1 expression and apoptosis of germ cells in mice testis. J Nutr Biochem 2007;18:553-64.
24. Heaton MB, Paiva M, Siler-Marsiglio K. Ethanol influences on bax translocation, mitochondrial membrane potential, and reactive oxygen species generation are modulated by Vitamin E and brain-derived neurotrophic factor. Alcohol Clin Exp Res 2011;35:1122-33.
25. Rahman SA, Anwar NA, Basha SS, Kamarzaman S, Shah JS, Mahkota BI. Impact of thymoquinone supplementation on immobilisation stress-induced changes in reproductive characteristics of male mice. J App Nat Sci 2014;1:1-5.
26. Abdallah MA, Kelany ME. Could thymoquinone protect the liver against indomethacin toxicity. Life Sci J 2014;11:26-33.
27. Khan UA, Aslam M, Saeed SA. Effect of beta adrenergic antagonist on the production of testosterone by rat's Leydig cells. J Ayub Med Coll Abbottabad 2004;16:26-8.
28. Kalra SP, Kalra PS. Opioid-adrenergic-steroid connection in regulation of luteinizing hormone secretion in the rat. Neuroendocrinology 1984;38:418-26.
29. Yilmaz B, Konar V, Kutlu S, Sandal S, Canpolat S, Gezen MR, *et al.* Influence of chronic morphine exposure on serum LH, FSH, testosterone levels, and body and testicular weights in the developing male rat. Arch Androl 1999;43:189-96.
30. Tawfeek FK, Ahmed SM, Kakel SJ. Effect of *Nigella sativa* oil treatment on the sex organs and sperm characters in rats exposed to hydrogen peroxide. Mesopotamia J Agric 2006;34:2-8.
31. El Khasmi M, Allah AI, Farh M, Riad F, Safwate A, El Abbadi N, *et al*. Effect of *Nigella sativa* fixed oil on the hormonal profile of androgens and circulating in male rats. Phytotherapie 2011;9:338-42.
32. Pinilla L, González LC, Tena-Sempere M, Bellido C, Aguilar E. Effects of systemic blockade of nitric oxide synthases on pulsatile LH, prolactin, and GH secretion in adult male rats. Horm Res 2001;55:229-35.
33. Weinberg JB, Doty E, Bonaventura J, Haney AF. Nitric oxide inhibition of human sperm motility. Fertil Steril 1995;64:408-13.
34. Stefano GB, Liu Y, Goligorsky MS. Cannabinoid receptors are coupled to nitric oxide release in invertebrate immunocytes, microglia, and human monocytes. J Biol Chem 1996;271:19238-42.
35. Jalili C, Salahshoor MR, Naseri A. Protective effect of *Urtica dioica* L against nicotine-induced damage on sperm parameters, testosterone and testis tissue in mice. Iran J Reprod Med 2014;12:401-8.
36. Guvvala PR, Sellappan S, Parameswaraiah RJ. Impact of arsenic(V) on testicular oxidative stress and sperm functional attributes in Swiss albino mice. Environ Sci Pollut Res Int 2016;23:18200-10.
37. Cho Ping N, Hashim NH, Hasan Adli DS. Effects of *Nigella sativa* (Habbatussauda) oil and nicotine chronic treatments on sperm parameters and testis histological features of rats. J Evid Based Complement Altern Med 2014;22;59-65.
38. Alhimaidi AR. Thymoqunone treatment of intracytoplasmic sperm injection (ICSI) compared to *in vitro* fertilization (IVF) of mice oocytes and their development *in vitro*. Adv Mol Med 2005;1:119-23.
39. Gil-Guzman E, Ollero M, Lopez MC, Sharma RK, Alvarez JG, Thomas AJ Jr., *et al.* Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. Hum Reprod 2001;16:1922-30.
40. Bolelli G, Lafisca S, Flamigni C, Lodi S, Franceschetti F, Filicori M, *et al.* Heroin addiction: Relationship between the plasma levels of testosterone, dihydrotestosterone, androstenedione, LH, FSH, and the plasma concentration of heroin. Toxicology 1979;15:19-29.
41. Kamarzaman S, Rahman SA. Effects of thymoquinone supplementation on cyclophosphamide toxicity of mouse embryo *In vitro*. Glob Vet 2014;12:80-90.