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Original Article

Evaluation of the Effect of Vitamin E on Reproductive Parameters in Morphine-Treated Male Mice

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Abstract

Background: Morphine is a narcotic pain reliever that is prescribed to reduce postoperative pain and can produce reactive oxygen species (ROS). Therefore, it can have negative effects on spermatogenesis and male fertility. Vitamin E is an effective antioxidant which plays an important role in membrane lipid peroxidation due to increased ROS. The present study aimed to evaluate the effects of vitamin E and morphine on sperm parameters, level of malondialdehyde (MDA), and diameter of seminiferous tubules in morphine-treated mice.

Methods: In this experimental study, 80 mice were divided into ten groups (n = 8) including control, normal saline, vehicle, morphine, various doses of vitamin E (100, 200, 300 mg/kg), and morphine plus vitamin E (100, 200, 300 mg/kg) groups. The groups were followed up for 30 consecutive days. Sperm parameters, testis weight, the diameter of seminiferous tubules, and the level of MDA were analyzed and compared.

Findings: Data analysis showed seminal parameters decreased significantly (excluding sperm count) and there was an increase in the level of MDA in morphine-treated mice compared with the normal saline group ($P < 0.05$). Administration of E100 to morphine-treated mice did not show a significant difference in the evaluated parameters compared with the morphine group. However, E200 and E300 significantly reduced MDA and improved sperm parameters ($P \leq 0.05$).

Conclusion: The results showed co-administration of vitamin E in high doses (200 & 300) could prevent the deleterious effects of morphine on some reproductive parameters and decrease the level of MDA in morphine-treated mice.

Keywords: Morphine, Male infertility, Sperm parameters, Malondialdehyde

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Introduction

Infertility is a global problem and epidemiological reports confirm approximately 15% of couples suffer from infertility, and men generally play a role in 50% of cases.[1] Various environmental, genetic, occupational, and pharmacological factors can be involved in causing this problem.

Morphine is an opioid analgesic drug that is prescribed to reduce postoperative pain. Unfortunately, its long-term use can lead to morphine antinociceptive tolerance (MAT) and the patient needs to take increasing doses to maintain analgesic effects.[2]

Morphine can change reactive oxygen species (ROS) production. ROS are highly reactive particles and can react with other biological molecules. In a study, it was shown that Hepatitis C virus (HCV) and Human immunodeficiency virus 1 (HIV-1) coexposed cells while exposure to morphine increased the ROS level significantly.[3]

Morphine has received much more attention in connection with oxidative stress (OS) than other drugs. In general, there are two conceivable ways by which this drug can contribute to OS. It seems that morphine is able to elevate the level of free radicals and decrease the antioxidant capacity in target cells. Both practical methods may be combined.[4]

Small amounts of free radicals are beneficial to the physiology of normal sperm, stimulate sperm capacitation, and increase the acrosome reaction and binding to zona pellucida, but as the level of ROS increases or the antioxidant activity decreases, the state of balance between the level of antioxidants and ROS is lost and OS occurs. Sperm cells are mainly susceptible to OS effects because they contain very small amounts of antioxidants that are not sufficient to protect them against high levels of ROS, hence the elevated level of ROS leads to lipid peroxidation in sperm plasma membranes and DNA fragmentation.[5]

Generally and in nature, the human body system creates a balance between free radical production and antioxidant capacity, but in a recent study, Osmanlıoğlu et al showed a decrease in oxidative enzyme levels and an increase in lipid peroxidation due to OS in rats exposed

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to morphine for a long time.[6] It has also been stated that morphine consumption can increase the number of free radicals in the body hence causing OS.[6]

Recent research has shown that fragmentation in sperm DNA is a very important factor in male infertility, and ROS can damage the DNA of sperm during its migration in male genital tube.[7] Evenson et al. showed that chromatin abnormalities such as DNA breakage in more than 30% of sperm can lead to male infertility.[8]

On the other hand, previous studies have confirmed the role of morphine in causing chromatin breakage in somatic cells in different parts of the body.[9,10] Today, there is a growing trend of oral antioxidant consumption to counteract the increased level of OS in the sperm and semen of infertile or subfertile men. This hypothesis is supported by several studies that describe the improvement of sperm parameters after taking oral antioxidants. Among these, improvements in sperm concentration and motility or reduced DNA damage have been reported.[11]

Several studies have shown that administration of oral antioxidants increases the scavenging capacity of ROS thus reducing the level of ROS in semen.[12,13] Vitamins are known as powerful antioxidants. Vitamin E is one of the most important antioxidant molecules located mainly in cell membranes; it plays a fundamental role in protecting the cell membranes against membrane lipid peroxidation due to increased ROS levels as well as trapping and inhibiting free radicals in cell membranes.[14] Vitamin E may control the production of ROS in two antioxidant-dependent and independent ways because it has shown its inhibitory effects on the activity and expression of ROS-producing enzymes.[15] In summary, vitamin E protects the cell membrane by keeping the glutathione level normal and inhibiting lipid peroxidation. It cleans the cells from high amounts of free radicals,[16,17] reducing the apoptosis level,[18,19] and possibly reducing OS and its negative effects on sperm membranes and DNA fragmentation. The antioxidant role of this vitamin in reducing testicular OS has been reported.[20,21]

A number of studies have shown positive effects of antioxidants on sperm parameters. Improving sperm parameters following vitamin E intake includes increasing sperm quality and quantity[22] and sperm survival percentage,[23] as well as maintaining sperm DNA integrity from OS. Moreover, vitamin E insufficiency can have negative effects on reproductive functions through damage of testicular tissue, decreased testosterone, and thus, reduced spermatogenesis.[24]

Due to the toxic effects of morphine to increase ROS levels, lipid peroxidation of cell membrane and malondialdehyde (MDA) concentration, as the final product of lipid peroxidation, as well as the properties of vitamin E to increase antioxidant enzymes, reduce ROS levels, and decrease MDA concentration, and since no study has yet been conducted in this area, the present study aimed to assess the effects of vitamin E on the level of MDA and some reproductive parameters in morphine-treated male mice.

Methods

This animal study was conducted on 80 adult NMRI male mice. The animals weighed 27–30 g (10 to 12 weeks) and were divided into ten groups. They were held at the animal house of Kurdistan University of Medical Sciences in similar conditions with a temperature of 22 ± 2°C, 12 hours light and 12 hours darkness in standard cages, fed with normal diet and water.

Experimental design

A total of 80 animals were divided into 10 groups (n = 8). The first group received morphine intraperitoneally. The morphine group received morphine once a day for 5 consecutive days (20 mg/kg), twice daily from the sixth to tenth days (20 mg/kg), and twice daily from the eleventh to twentieth days (30 mg/kg).[25] Groups 2 (100 mg/kg), 3 (200 mg/kg), and 4 (300 mg/kg) received vitamin E. Mice with vitamin E plus morphine including group 5 [E100 + morphine (20 mg/kg)], group 6 (E 200 + morphine), group 7 (E 300 + morphine), and group 8 did not receive any treatment, group 9 received 0.9% normal saline, and group 10 or vehicle group received olive oil (0.1 mL/10 g body weight).[26] All groups were treated for 30 consequent days.

MDA measurement

Sperm cells are more sensitive against OS and elevated levels of free radicals can create lipid peroxidation in sperm plasma membranes.[5] Besides, morphine has received much more attention in connection with OS than other drugs. On the other hand, MDA is one of the end products of the peroxidation of polyunsaturated fatty acids and the increase in the level of OS is responsible for MDA overproduction.

The basis of this method is the reaction of MDA with thiobarbituric acid (TBA) and the absorbance is measured at 535 nm.

First, the mice were anesthetized with ketamine, and then blood samples were taken from their hearts with a needle to asses MDA levels. The plasma of the blood samples was separated using a centrifuge (4000 rpm for 10 minutes) and stored at –70°C until the MDA concentration was measured.

After thawing, 200 µL of plasma was mixed with 1.5 mL TBA, 1.5 mL glacial acetic acid, and sodium dodecyl sulfate. All samples containing the mixture were heated at 100°C for 1 hour. After cooling, the absorbance was read at 532 nm.

Testicular weight measurement

Cervical dislocation was performed for animal

scarification. After that, the epididymis (caudal section) was placed into a lab dish. Then, the testicles were removed from the body and weighed with a microbalance sensitive to 0.001 mg (Precise 125A, Switzerland).

Evaluation of sperm parameters

Sperm analysis was done according to the 5th edition of World Health Organization (WHO) guideline. Sperm count, motility (motile and immotile percentages), normal morphology, and viability were assessed.[27]

Sperm count

Briefly, the dissected epididymis was transferred into Ham's F10 medium. A small amount of the suspension was mixed with an equal amount of 10% formaldehyde (Sigma, USA). Then, 10 µL of this mixture was transferred to a pre-heated Neubauer hemocytometer and covered with a cover slip. After seven minutes, the sperms were observed, counted, and recorded by an optical microscope in 4 fields (×400).

Sperm viability

Eosin Y staining was used to evaluate the viability of sperms. To this end, 10 µL fresh sperm suspension was mixed with 10 µL 1% eosin on a glass slide. A smear was prepared and allowed to air dry. At least 250 sperms were evaluated from each sample by a light microscope (×400). After staining, dead and live sperms appeared pink and white, respectively.

Sperm morphology

Diff Quick staining was used to evaluate sperm morphology. A thin smear of 10 µL fresh sample was prepared and allowed to dry for about 15 minutes. After staining, 200 spermatozoa were evaluated on each slide by light microscopy (×100).

Sperm motility

For motility evaluation, 10 µL from each sperm sample was placed on a chamber slide. At least 200 spermatozoa were evaluated in five different regions by an optical microscope at ×400. Finally, sperm motility was classified as progressively motile (fast: A, slow: B), non-progressive (C), and immotile (D).

Diameter of seminiferous tubules

After fixing the sperm tissue samples with 10% formaldehyde, they were dehydrated by graded alcohols in ascending order and clarified with xylene and embedded in paraffin.

Microscopic sections (5 microns) were prepared from the samples and stained with hematoxylin and eosin staining. Then, 20 cross-sections of seminiferous tubules were randomly selected from eight mice in each experimental group. The average diameter of tubules was measured for each testicle separately.[28]

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (version 16.0, SPSS Inc., Chicago, IL, USA). First, the data distribution was normalized using the Kolmogorov-Smirnov test. Then, two-way analysis of variance (ANOVA) as well as Tukey HSD post-hoc test were used to compare the effects of two factors (addiction and treatment) on study parameters between groups *(P* value < 0.05).

Results

Sperm analysis was done in all groups and acquired data are summarized in Tables 1 and 2. The results did not show a significant difference in the evaluated factors between the control, normal saline, and vehicle groups. Moreover, in comparison with the saline group, the morphine-treated group showed a remarkable decrease in the sperm total motility, viability, normal morphology, seminiferous diameter, and testis weight and a significant increase in the level of MDA. Compared to M + E200, M + E300 showed a significant increase, while no significant difference was observed between the other groups (Tables 1 and 2).

Sperm motility

Total motility

Compared to the morphine group, M + E300 and M + E200 groups showed a significant increase ($P = 0.000$) and compared to the control group, E300 and E200 groups revealed a significant increase ($P = 0.000$). Moreover, there was a significant increase in M + E200 compared to the M + E100 group, in M + E300 compared to the M + E200 group, and in E200 compared to the E100 group ($P = 0.000$). Furthermore, the results showed that E300 compared to the E200 group had a decrease in total motility, but this decrease was not statistically significant (Tables 1 and 2).

Types of motility

There was a significant decrease in A and B types of motility in morphine compared to the normal saline group but there was a significant increase in D group ($P = 0.000$). The obtained results also showed a significant increase in A and B motility in M + E200 and M + E300 groups compared to the morphine group, as well as in E300 and E200 compared to the control group. Type A of motility showed a significant increase in E200 in comparison to the E100 group but type C showed a significant decrease ($P = 0.000$) (Tables 1 and 2).

Sperm viability

There was a significant increase in M + E300 and M + E200 compared to the morphine group and in E200 compared to the control group. The results also showed that the

Table 1. Examined factors (sperm parameters, MDA, seminiferous diameters and testis weight) in experimental groups

Factors	Control	Normal saline	Olive oil	Morphine	M+E100	M+E200	M+E300	E100	E200	E300
Count (10^6)	37.12±2.23	37.00±1.92	37.5±2.07	34.75±1.98	36.75±3.15	36.87±2.47	37.12±2.03	38.47±1.28	39.55±3.49	37.55±1.46
Total motility (%)	73.25±3.61	73.5±1.41	74.75±1.66	42.87±2.03	43.0±2.33	68.87±2.47	68.62±3.58	73.62±2.66	80.62±1.30	79.12±1.64
Progressive motility										
Fast %	18.25±2.12	18.12±2.35	19.12±2.23	0.00±0.00	0.5±0.75	15.62±2.13	17.50±1.60	19.87±0.99	31.00±1.30	29.62±1.59
Slow %	21.87±2.03	22.87±1.80	22.75±2.91	11.75±2.12	14.25±2.37	23.87±3.18	23.50±3.25	28.75±4.46	27.25±4.09	25.62±1.84
Non-progressive motility %	32.50±2.61	32.50±2.00	31.37±1.30	31.12±2.79	28.75±3.37	29.87±1.80	27.62±2.38	25.12±2.23	23.75±3.19	23.87±2.85
Immotile %	26.75±3.61	26.50±1.41	25.25±1.66	57.12±2.03	56.25±2.76	30.87±2.69	30.12±4.54	26.25±2.54	20.37±3.24	20.87±1.64
Viability %	76.12±2.94	76.00±1.06	74.5±2.25	65.5±2.50	55.5±3.74	70.87±2.35	71.75±3.28	77.5±2.97	83.00±1.77	80.37±1.50
Normal morphology %	67.62±4.27	67.00±4.98	65.50±4.37	33.75±2.12	34.62±1.50	57.12±3.04	56.37±3.54	66.75±4.52	71.00±2.72	67.00±4.03
MDA	3.137±0.09	3.16±0.03	3.18±0.08	4.40±0.1	4.38±0.06	3.20±0.07	3.13±0.07	3.16±0.05	3.07±0.06	3.18±0.04
Seminiferous diameter (μm)	170.62±0.9	171.49±1.25	171.59±1.03	162.94±0.82	167.0±1.41	172.12±0.69	180.81±0.75	173.12±0.69	179.69±o.70	171.62±0.74
Testicle weight (g)	188.38±6.23	192.38±21.65	190.12±6.72	171.50±9.31	203.12±28.11	211.62±14.17	213.04±21.31	174.62±2.15	236.5±29.09	191.50±24.08

Note: MDA: Malondialdehyde, M: Morphine, E: Vitamin E

Two-way analysis of variance (ANOVA) tests were performed to determine the statistical significance between different groups. Data are expressed as mean±SD.

Table 2. Comparison of sperm parameters, MDA, seminiferous diameter, and testicle weight in experimental groups

Factors	*P* Values Between Groups												
	Control & Normal Saline	Control & Olive Oil	Morphine & Normal Saline	Morphine & M+E100	Morphine & M+E200	Morphine & M+E300	Control & E100	Control & E200	Control & E300	E100 & E200	E200 & E300	M+E100 & M+E200	M+E200 & M+E300
Count	0.922	0.769	0.081	0.121	0.099	0.066	0.292	0.061	0.713	0.401	0.653	0.922	0.013*
Total motility	0.837	0.217	0.000*	0.918	0.000*	0.000*	0.756	0.000*	0.000*	0.000*	0.217	0.000*	0.000*
Progressive motility													
Fast	0.882	0.299	0.000*	0.552	0.000*	0.000*	0.056	0.000*	0.000*	0.000*	0.105	0.000*	0.000*
Slow	0.500*	0.555	0.000*	0.094	0.000*	0.000*	0.000*	0.001*	0.013*	0.312	0.274	0.000*	0.800
Non progressive motility	1.000	0.377	0.281	0.065	0.327	0.007*	0.000*	0.000*	0.000*	0.281	0.923	0.377	0.08
Immotile	0.858	0.284	0.000*	1.000	0.000*	0.000*	0.720	0.090	0.000*	0.000*	0.720	0.000*	0.591
Vitality	0.923	0.902	0.000*	0.438	0.000*	0.000*	0.287	0.000*	0.235	0.000*	0.028*	0.000*	0.000*
Normal morphology	0.735	0.251	0.000*	0.635	0.000*	0.000*	0.635	0.071	0.735	0.024*	0.033*	0.000*	0.684
MDA	0.455	0.214	0.000*	0.226	0.000*	0.000*	0.533	0.122	0.235	0.032*	0.000*	0.000*	0.064
Seminiferous diameter (μm)	0.068	0.068	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.037*	0.000*	0.000*	0.000*	0.000*
Testicle weight (g)	0.7	0.866	0.047*	0.739	0.000*	0.005*	0.213	0.000*	0.763	0.001*	0.001*	0.414	0.056

Note: MDA: Malondialdehyde, M: Morphine, E: Vitamin E.

Two-way analysis of variance (ANOVA) and Tukey HSD post-hoc tests were performed to determine the statistical significance between different groups. $P \leq 0.05$ is considered significant.

E200 group had a significant increase in sperm viability compared to the E100 group (Tables 1 and 2).

There was a statistically significant increase in E200 compared to the E100 group. The results also showed a significant increase in E200 compared to the E100 group ($P=0.00$) but vitamin E300 caused a statistically significant decrease in sperm vitality compared to E200 ($P=0.033$) (Tables 1 and 2).

Sperm normal morphology

Administration of E200 and E300 resulted in a significant increase in sperm normal morphology in the morphine-treated mice ($P=0.000$). There was a significant increase in normal morphology in E200 compared with the E100 group ($p=0.024$) but E300 had a significant decrease in comparison to the E200 group ($P=0.033$) and M + E200 had a significant increase compared to the M + E100 group (Tables 1 and 2).

Seminiferous diameter

A statistically significant increase was observed in the diameter of tubules in all groups except for the E300 group, which showed a significant decrease compared to the E200 group ($P=0.000$) (Tables 1 and 2).

Testicle weight

Administration of E200 and E300 resulted in a significant increase in the testis weight in the morphine-treated mice and E200 led to a statistically significant decrease in the weight of the testicles compared to the control group. There was a significant increase in the testis weight in E200 in comparison to the E100 group ($P=0.001$) but E300 showed a significant decrease compared to the E200 group ($P=0.001$) (Tables 1 and 2).

Malondialdehyde

Administration of E200 and E300 resulted in a significant decrease in the level of MDA in the morphine-treated mice and E200 showed a significant decrease in comparison to the control group ($P=0.00$). A significant decrease was observed in the level of MDA in M + E200 in comparison to the M + E100 group ($p=0.000$) and in E200 compared to the E100 group ($P=0.032$); however, E300 in comparison to E200 revealed a significant increase ($P=0.000$) (Tables 1 and 2).

Discussion

Opioids, including morphine, are among the most effective and powerful drugs used to control pain,[29] but they also reduce male fertility by affecting sperm parameters.[9] The effects of vitamin E on the sperm parameters, testicular weight, diameter of seminiferous tubules, and lipid peroxidation in morphine-treated mice were investigated in the present study.

The results of this study showed morphine administration reduced sperm total motility, viability, normal morphology, seminiferous diameter, and testicle weight and increased the level of MDA, but sperm count did not change. The results obtained from a number of studies revealed that morphine consumption has a role in reducing the quality of sperm parameters and the weight of sexual organs.[25,30] In a case-control study on 30 men abusing morphine, Ghasemi-Esmailabad et al showed a significant decline in progressive and total motility in comparison to the control group and concluded that morphine dependence reduces male fertility by affecting sperm parameters.[9] The results of the present study showed a significant decrease in total motility and fast and slow progression as well as an increase in immotile sperm cells.

Cai et al reported that morphine activates μ-opioid receptor (MOR), resulting in the production of ROS[31] and another study reported an increase in nitric oxide.[25] ROS production due to morphine and reduced activities of antioxidant enzymes can lead to oxidative damage to various biomolecules such as lipids, proteins, and DNA.[32]

Morphine administration led to a significant increase in the MDA level. MDA is an indicator and metabolite of lipid peroxidation and it is also one of the final products of the effect of superoxide anion on unsaturated fatty acids. A negative correlation has also been reported between MDA level and sperm parameters such as morphology and motility.[33] In fact, the increase in ROS causes membrane lipid peroxidation, which leads to the formation of MDA.

Sperm membranes are high levels of unsaturated fatty acids, including docosahexaenoic acid, which have six double bonds per molecule, thus being very sensitive to ROS-induced oxidation.[34] Therefore, the increase in the level of ROS in morphine-treated mice may be the reason for the decrease in the number of sperm parameters, the diameter of seminiferous tubules, and the weight of testicles.

A balance between free radicals and antioxidant levels is necessary to inhibit OS. Antioxidants can counteract with free radicals and neutralize oxidants, thus protecting the cell against OS. Vitamin E is a fat-soluble and non-enzymatic antioxidant which is able to reduce lipid peroxidation in spermatozoa.[35]

The present study evaluated the dose-dependent protective effects of vitamin E (E100, E200, E300) on the morphine-treated male mice in terms of total motility (types of motility: A, B, C, D), normal morphology, sperm count, vitality, testis weight, the diameter of seminiferous tubules, and the level of MDA.

The results showed no significant difference in the MDA level between M + E100 and the control group and the lack of reduction in the level of MDA in M + E100 compared to the morphine group might be the reason for the lack of improvement in sperm parameters in this

group. However, it seems that higher doses (E200 and E300) inhibited lipid peroxidation and decreased MDA.

Moreover, the present study indicated that E200 and E300 improved the reproductive parameters assessed in morphine-treated mice. This is probably due to the scavenging property of free radicals by vitamin E. Vitamin E may also be able to increase the production of antioxidant enzymes.[36]

Other studies have measured the effect of vitamin E on reproductive system under other conditions. Vitamin E treatment in diabetic mice improved the sperm total motility, normal morphology, apoptosis, and total antioxidant capacity but MDA concentration was similar in all groups.[37] Coadministration of mancozeb-treated pups with 200 mg/kg vitamin E led to a reduction in the toxic effects of mancozeb on sperm vitality, number, motility, morphology, and testis structure.[26]

Vitamin E (100 mg/kg/d) significantly improved sperm parameters in sodium arsenite-treated rats.[38] On the other hand, in a double-blind study, 124 male patients with diagnosed infertility were supplemented by 400 IU vitamin E daily for 56 days. There was no significant difference in sperm parameters and fertility, but a significant decrease was observed in normal morphology sperms in the placebo group. It was stated that vitamin E may have been able to prevent the harmful effects of OS on sperm and additional studies were recommended to assess the effect of various doses of vitamin E.[39]

In another double-blind randomized study, for 90 days, 400 mg/d vitamin E was given to the male partner of infertile patients who were undergoing in vitro fertilization (IVF). It was found that vitamin E administration could significantly increase sperm progressive motility in the treated group in comparison with the placebo group. However, vitamin E administration significantly increased the live birth rate and improved the results of other IVF parameters.[40]

Administration of vitamin E to 45 patients undergoing varicocelectomy for 12 months non-significantly improved all sperm parameters.[41] Oral treatment with vitamin E in adult rats treated with para-nonylphenol, significantly increased sperm motility and viability.[42] In vitro use of alpha-tocopherol in teratozoospermic men indicated that vitamin E can increase sperm viability and motility.[43]

Several studies have investigated the effect of vitamin E on other tissues, highlighting its role in further reducing lipid peroxidation. In a study, Jain et al. found that glutathione levels were significantly related to vitamin E levels and daily consumption of 100 IU vitamin E significantly elevated glutathione levels and reduced peroxidation of lipid in erythrocytes in diabetic cases. Glutathione is also a cofactor for a number of enzymes that catalyze the detoxification of intracellular peroxides.[44]

In another study, it was found that high doses of vitamin E (200 mg/kg) can protect red blood cells from the OS harmful effects by decreasing the level of lipid peroxidation and increasing the antioxidant activity.[45]

The present study also showed reproductive parameters significantly improved while MDA levels decreased in M + E200 compared to the M + E100 group. Sperm total motility, fast progressive motility, and sperm viability increased significantly in M + E300 compared to the M + E200 group, but normal morphology and MDA decreased non-significantly.

Furthermore, the comparison between vitamin E groups showed a significant improvement in sperm parameters in E200 (group 3) and E100; but interestingly, in E300 (group 4), the sperm parameters decreased and MDA concentration non-significantly increased in comparison to E200 (group 3).

A number of studies have been conducted on the effect of vitamin E on different populations, which have reported different results. El-Hak et al investigated the effect of different doses of vitamin E on male albino rats. They found that E500, E1000, and E2000 mg/kg body weight administered for 30 consequent days were not safe for the kidney, liver, and testes.[46] Another study investigated the protective effects of vitamin E on sperm DNA fragmentation, quality of chromatin, and a number of sperm parameters in mice treated with different doses of ethanol. Their results showed that E100 and ethanol 10% plus vitamin E200 groups had the highest protective effect.[47] Wang et al showed vitamin E (45, 150, and 500 mg/kg/d) could be used as a prophylactic agent to prevent bisphenol A-induced reproductive toxicity in male Kunming mice.[48] The results of another study showed administration of vitamin E (200 mg/kg) can decrease the adverse effects of MDA on sperm parameters.[26]

It is necessary to evaluate the clinical safety and toxicity of vitamin E in different populations. Accordingly, further studies are recommended to more accurately evaluate the appropriate dose of vitamin E in the morphine-treated group.

Conclusion

The present study indicated that morphine decreased sperm parameters and increased the level of MDA. Co-administration of vitamin E to morphine-treated mice improved reproductive parameters and reduced the level of MDA, based on the dose consumed, which is probably due to the scavenging property of ROS. Moreover, E200 improved reproductive parameters compared to E100, but interestingly, E300 non-significantly reduced reproductive parameters compared to E200. Future studies are required to confirm the results of this study with different vitamin E dosages.

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Competing Interests

The authors declared no conflict of interest.

Ethical Approval

All experimentation was performed under the approval of the ethics committee of Kurdistan University of Medical Sciences (Ethical code: IR.MUK.REC.1399,302).

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