

Melatonin Improve the Sperm Quality in Forced Swimming Test Induced Oxidative Stress in Nandrolone Treated Wistar Rats

Bagher Minaii¹, Ardeshir Moayeri^{1,2}, Saeed Shokri³, Mehryar Habibi Roudkenar⁴,
Taghi Golmohammadi⁵, Fatemeh Malek¹, and Mohammad Barbarestani¹

¹ Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

² Department of Anatomy, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

³ Department of Anatomy, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

⁴ Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

⁵ Department of Clinical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Received: 10 Jun. 2013; Received in revised form: 15 Jun. 2013; Accepted: 16 Jun. 2013

Abstract- This study investigates the effects of melatonin on the sperm quality and testis weight after the combination of swimming exercise and nandrolone decanoate (DECA). Two groups of male Wistar rats were treated for eight weeks as follows; group A consist of CO (control), Sham, N (DECA), S (swimming) and NS (DECA plus swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin plus DECA), MS (melatonin plus swimming), MNS (melatonin, DECA plus swimming). The motility of sperm was significantly improved in melatonin groups in comparison to N, S and NS groups ($P \leq 0.05$). The left testes weight was decreased in N, NS and MNS groups, and the right testes weight was decreased in N,S,NS, MS and MNS groups in compare with the control group. This study concluded that melatonin probably could improve the sperm motility and sex organs weight after the combination of DECA and exercise.

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Acta Medica Iranica, 2014;52(7):496-504.

Keywords: Melatonin; Nandrolone Decanoate; Swimming; Testis; Rat

Introduction

Anabolic-androgenic steroids (AAS), misuse to progress muscle mass or athletic capability, ranks among the most widely abused drugs (1). Unfortunately, in the last decay the abuse of these agents has significantly increased in all around the world. The combinations of these compounds abused by athletes are typically in excess of therapeutic doses (10- to 100-times) as derivatives of testosterone, anabolic steroids seriously affect the male pituitary-gonadal axis (2).

Misuse AAS may be an etiological issue in male infertility among leisure power athletes. Both exogenous androgens and exercise are known to apply subclinical alterations in male reproductive tissues (2-4). In the last project, we showed that the combination of nandrolone decanoate (DECA) and exercise can dramatically reduce the sperm quality and sex organs weight (1).

Some studies showed that the exercise increases the oxygen consumption rate by 10–20 times, resulting in an enhanced production of reactive oxygen species (ROS) in

testes and/or sperm. ROS are free radicals such as the hydroxyl radical (OH) and the superoxide anion (O_2^-) which can increase the rates of cellular death (5). The making of ROS is a normal physiological incident in various organs, including the testes. On the other hand, the overproduction of ROS stimulates DNA fragmentation that can be harmful to sperm function, associated with peroxidative damage to the mitochondria and plasma membrane. Last project we showed that the combination of Swimming exercise and nandrolone can disturb the sperm function and DNA integrity (1).

Some studies demonstrated that swimming could depress melatonin content in the rat pineal gland (6). The pineal hormone melatonin (N-acetyl-5-methoxytryptamine) as a very potent antioxidant can easily cross cell membranes and the blood-brain barrier (7). Once synthesized in the pineal gland, melatonin is quickly free into the blood stream and then into other body fluids, such as cerebrospinal fluid, bile, ovarian follicular fluid, semen, saliva, and amniotic fluid (8,9). Melatonin is exerting a regulatory role at different levels

Corresponding Author: S. Shokri

Department of Anatomy, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
Tel: +98 241 424 0279, Fax: +98 241 424 9553, E-mail address: saeedshus@zums.ac.ir

of the hypothalamic–pituitary–gonadal axis (10). It has been shown to protect the cell against oxidative stress in highly, a range of contrary experimental systems. In actual, the chemical property of melatonin easily and efficiently scavenge several oxygen free radicals (11, 12). Moreover, the early investigations have already presented pharmacological and physiological concentrations of melatonin could preserve DNA from damage by free radicals (13,14). Melatonin also plays an important role in guarded the testes from oxidative stress. The functions of melatonin about reproductive physiology are known as well (15).

Low endogenous melatonin levels of semen are associated with an increased prevalence of abnormal sperm progression (16). Exogenous or endogenous stresses such as exercise and AAS can affect the sperms while developing process. Melatonin receptors have been found in the epididymis, and low-affinity melatonin-binding sites have been showed on spermatozoa (17). Therefore, it is possible that melatonin can control sperm motility, when spermatozoa develop through the epididymis (18).

Melatonin as a powerful antioxidant probably is able to improve some oxidative stress induced by a combination of exercise and high dose of AAS in testes and/or sperm. On the other hand, the combined effects of melatonin, swimming training and supraphysiological dose of exogenous androgens on spermatogenic cells have not been investigated simultaneously. As a whole, the present study was conducted to evaluate the effects of exercise and weekly injections of melatonin and DECA for eight weeks on the sperm quality and sex organs weight in the rats model in order probably to shed some light in the improving male infertility in athletes that abuse AAS (19).

Materials and Methods

Animal procedure

These experiments were carried out, in accordance with the national guidelines and protocols for the care and use of laboratory animals, approved by the Institutional Animal Ethical Committee (IAEC no. 03 / 028 / 07). Healthy adult male Wistar strain rats weighing 170–200 g (3 months old) were randomly selected from the School of Pharmacy Laboratory Animal Center of the Tehran University of Medical Sciences. The animals were used and housed in a specific pathogen-free environment and animal house they were placed in standard rat cages under a 12-hr light: dark cycle with a room temperature of 23– 25°C. The rats were fed a

standard laboratory diet (Pars dam factory, Tehran, Iran) and clean drinking water was available.

Exercise design

In this study, swimming was considered as a suitable form of endurance exercise training as previously published by Shokri *et al.*, (1). Briefly, the swimming procedure included adaptation and training phases. The adaptation phase took six days; the exercise period was increased by 10 minutes each day until the rats could swim for 60 minutes. The training phase was engaged for one hr of continuous swimming per day, 5 days a week for eight weeks, (between 11:00 and 13:00 o'clock on each training day). Exercise was performed by swimming in an iron tank (length 137.5 cm, width 135 cm, depth 50 cm) containing tap water maintained at 32–34°C. A maximum of only 10 rats was permitted to swim together.

Treatment and chemicals

In this study, DECA or nandrolone decanoate (25 mg/ml) was purchased from Iran Hormone Company (Tehran, Iran) and Melatonin (Sigma Chemical Co., St. Louis, MO) was purchased from USA Sigma Company, and Negrosin was purchased from Germany Roche Company. The rats were randomly divided into two big different groups (n=12 rats each subgroup). The subgroups of the group A consist of: (Sham); rats received the solvent of DECA or peanut oil as a vehicle, Control (CO); without any injection or exercise protocol, (N); rats received 10 mg/kg/weekly of DECA for eight weeks, (S); the exercise protocol was employed for one hour continuous swimming per day, five days a week for eight weeks (1,20), (NS); rats received 10 mg/kg weekly DECA, and the exercise protocol was employed as S group. The subgroups of group B consist of: Melatonin (M); was dissolved in ethanol and further diluted in saline to give a final concentration of 1%, and rats received 10mg/kg weekly (IP or intra peritoneal) of melatonin for 8 weeks, (MN); they received melatonin (10mg/kg) and DECA (10 mg/kg), (MS); rats received melatonin (10mg/kg) and swimming exercise was employed, (MNS); they received melatonin (10mg/kg), DECA (10 mg/kg) and swimming protocol was employed, (ShamM); rats received the solvent of melatonin (ethanol 1%) as a vehicle.

Laboratory studies

Body and reproductive organ weights: Difference between initial and final body weight divides to summation of these two weights multiply 100 was

defined as the percentage changes in body weight for each group (21). After weighing, animals were anaesthetized with a combination of ketamine (45 mg/kg) and xylazine (35 mg/kg) intraperitoneally. Then, testes were cautiously dissected out, cleaned of fat and adhering connective tissue then accurately weighed. The organ to body weight ratio multiply 100 were calculated as a relative wet weight of organs.

Assessment of sperm characteristics: Epididymal sperms were composed by chopping the caudal part of epididymis in five ml of Ham's F10 solution and incubated for 15 minutes at 37°C to permit sperm to swim out of the epididymal tubules. After pipetting One drop of sperm suspension was located on a microscope slide, and a cover slip was placed over the slide. By the side of slightest 10 microscopic fields were investigated at 400× magnification using a light microscope, and the percentage of motile, progressive and immotile sperm was expressed as a fraction of the total counted sperm according to the WHO recommendations. The epididymal sperm counts were obtained by the technique explained in the WHO recommendation (22).

In brief, a 5µl aliquot of epididymal sperm was thinned with 95µl of diluents (0.35% formalin containing, 5% NaHCO₃ and 0.25% trypan blue) and approximately 10µl of this diluted specimen was changed to each of the counting chambers of the haemocytometer, which was allowed to stand for 5 minutes in a moist chamber to avoid drying. The cells were sediment during this time and were calculated with a light microscope at 400× magnification. For analyzing sperm viability, 20 microliters of sperm suspension were mixed with an equal volume of 0.05% eosin-Y. After two minutes, incubation at room temperature, slides were viewed by bright-field microscope. Dead sperms become visible pink and Live sperms were not stained (23).

Two hundred sperms were numbered for each model, and viability percentages were calculated. Finally, Sperm smears were drawn on a clean slide for the analysis of morphological abnormalities, and allowed to dry in air during the overnight. The slides were stained with 5% nigrosin-1% eosin Y and checked at 400× magnification for morphological abnormalities such as bending neck, tailless, bending tail, coiled or multiply abnormality (23).

Statistical analysis

Results were analyzed using the SPSS version16. All data were expressed as mean values and their standard errors (SE). The variables were analyzed by one-way

analysis of variance (anova) with melatonin, DECA and exercise as factors. When a significant co-administration effect was found, Tukey post hoc test was performed. The statistical significance level was set at $P \leq 0.05$.

Results

Body weight change and organ weight

The effects of melatonin, exercise and DECA treatments on the body weight change and relative accessory reproductive organ weight are shown in Table 1. Statistically significant differences in the body weight change were observed among some groups (N, S, NS, MN, and MNS) in comparison to those of the control and sham groups.

The mean right testes weight in N, S ($P \leq 0.01$, Figure 6), NS, ($P \leq 0.0001$, Figure 6) MS, MNS groups ($P \leq 0.5$, Figure 6) significantly decreased comparison to the control and sham groups. The mean left testes weight in the N ($P \leq 0.5$, Figure 7), MNS ($P \leq 0.01$, Figure 7), NS ($P \leq 0.0001$, Figure7) significantly decreased in comparison to those of the control and sham groups.

Table 1. Effects of melatonin on the body weight changes after the coadministration of swimming exercise and supraphysiological dose of nandrolone decanoate in mature male rats

Groups	Body weight changes (%)
Control	10.73±2.27
Sham	10.11±4.57
N	4.53±3.07 ^{a,b}
S	4.53±4.69 ^{a,b}
NS	2.73±2.3 ^{a,b}
Sham+M	10.39±2.19
M	10.76±4.76
MN	4.75±1.82 ^{a,b}
MS	8.62±2.48 ^d
MNS	4.53±0.84 ^{a,b}

Values are mean ± SD. N=12

^a $P \leq 0.05$ versus Control group.

^b $P \leq 0.05$ versus sham group.

^d $P \leq 0.05$ S group versus MS group

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

Epididymal sperm parameters

Statistically, decrease in total epididymal sperm count was observed in N, S, NS, MN, MS and MNS groups in comparison to the control and sham groups (Figure 1). Mean progressive motility of sperm in the N, S, NS, ($P \leq 0.0001$), MN, MNS ($P \leq 0.001$) and MS ($P \leq 0.01$) groups significantly decreased in comparison to those of the control and sham groups (Figure 2). Furthermore, Figure 3 shows that the total motility of sperm has

significantly decreased dramatically in the NS, MNS ($P \leq 0.0001$), N ($P \leq 0.001$), S ($P \leq 0.01$), MN ($P \leq 0.05$) groups in comparison to those of the control and Sham groups. Figure 4 shows the mean sperm viability in the N, S, NS, MNS, MS ($P \leq 0.0001$) and MN ($P \leq 0.001$) groups significantly decreased in comparison to the

control and sham groups. In addition, Figure 5 shows that the mean number of morphologically normal sperm in NS ($P \leq 0.0001$), N, S, MN, MNS ($P \leq 0.001$) MS ($P \leq 0.01$) groups significantly decreased compare with those of the control and sham groups.

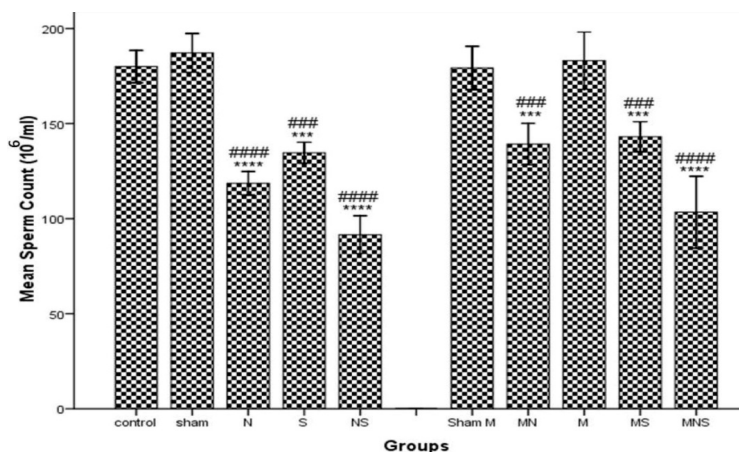


Figure 1. Mean sperm count of rats in control, sham and experimental groups

Values are mean \pm SE, N=12

***p \leq 0.001 versus Control-operated animals.£

****p \leq 0.0001 versus Control-operated animals.

###p \leq 0.001 versus sham-operated animals.

####p \leq 0.0001 versus sham-operated animals.

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

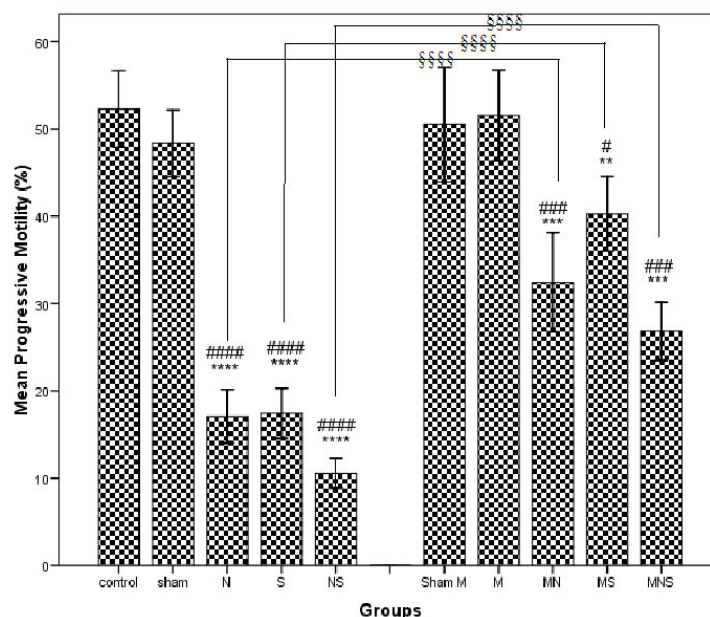


Figure 2. Mean progressive motility of sperm in control, sham and experimental groups.

Values are mean \pm SE, N=12

**p \leq 0.01 versus Control-operated animals

***p \leq 0.001 versus Control-operated animals.

****p \leq 0.0001 versus Control-operated animals.

#p \leq 0.05 versus sham-operated animals.

###p \leq 0.001 versus sham-operated animals.

####p \leq 0.0001 versus sham-operated animals

§§§§ p \leq 0.0001 between experimental groups (N vs. MN, S vs. MS and NS vs. MNS)

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

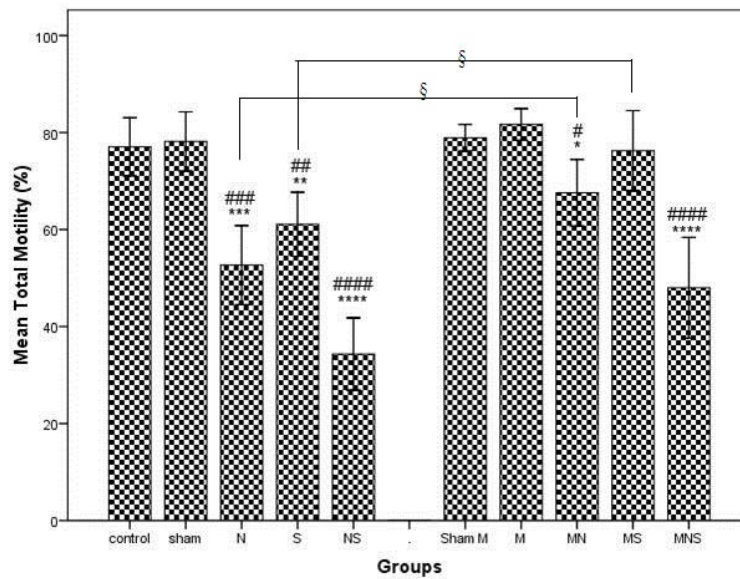


Figure 3. Mean total motility of sperm in control, sham and experimental groups.

Values are mean ± SE, N=12

*p ≤ 0.05 versus Control-operated animals

**p ≤ 0.01 versus Control-operated animals

***p ≤ 0.001 versus Control-operated animals.£

****p ≤ 0.0001 versus Control-operated animals.

##p ≤ 0.01 versus sham-operated animals.

###p ≤ 0.001 versus sham-operated animals.

####p ≤ 0.0001 versus sham-operated animals

§ p ≤ 0.05 between experimental groups (N vs. MN and S vs. MS)

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

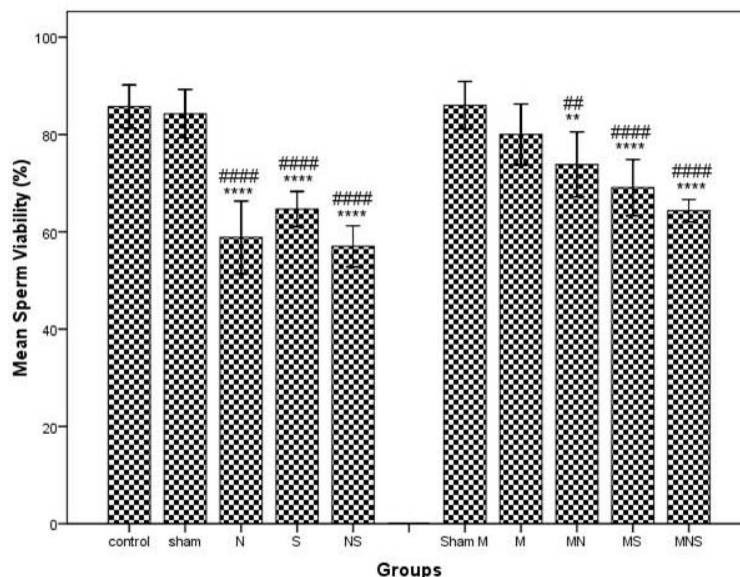


Figure 4. Mean sperm viability of rats in control, sham and experimental groups.

Values are mean ± SE, N=12

**p ≤ 0.01 versus Control-operated animals

***p ≤ 0.0001 versus Control-operated animals.

##p ≤ 0.01 versus sham-operated animals.

####p ≤ 0.0001 versus sham-operated animals

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

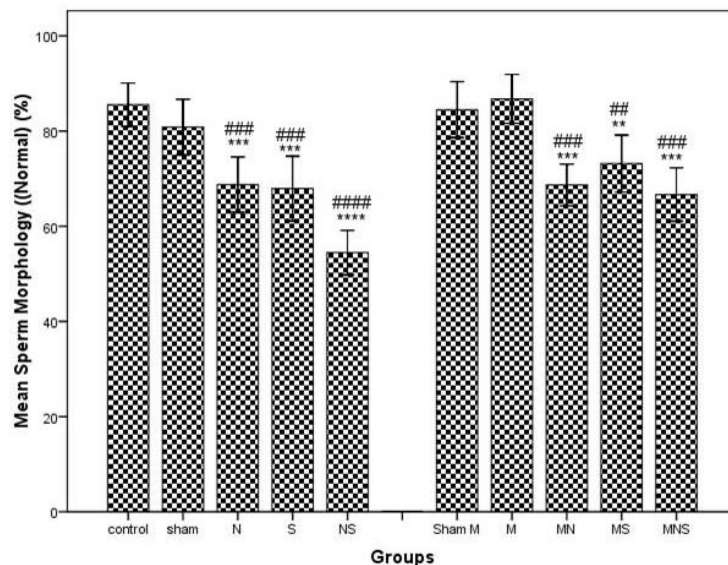


Figure 5. Mean sperm morphology (normal) in control, sham and experimental groups.

Values are mean ± SE, N=12

**p ≤ 0.01 versus Control-operated animals

***p ≤ 0.001 versus Control-operated animals.

****p ≤ 0.0001 versus Control-operated animals.

##p ≤ 0.01 versus sham-operated animals.

###p ≤ 0.001 versus sham-operated animals.

####p ≤ 0.0001 versus sham-operated animals

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

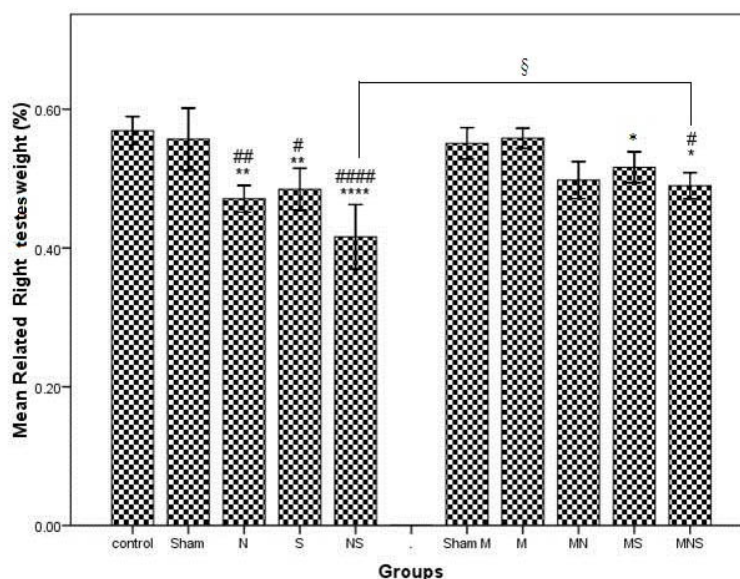


Figure 6. Mean relative right testis weight in control, sham and experimental groups.

Values are mean ± SE, N=12

*p ≤ 0.05 versus Control-operated animals

**p ≤ 0.01 versus Control-operated animals

***p ≤ 0.0001 versus Control-operated animals.

#p ≤ 0.05 versus sham-operated animals.

##p ≤ 0.01 versus sham-operated animals.

####p ≤ 0.0001 versus sham-operated animals

§ p ≤ 0.05 between experimental groups (N vs. MN and S vs. MS)

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

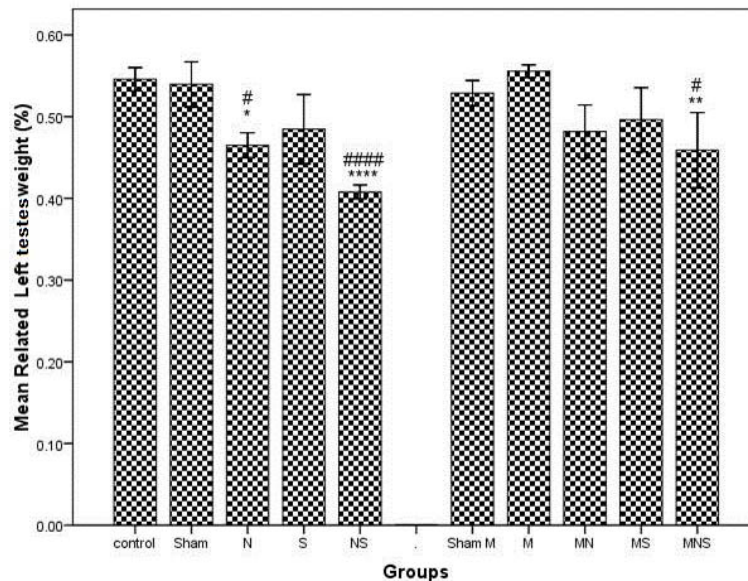


Figure 7. Mean relative left testes weight in control, sham and experimental groups.

Values are mean \pm SE, N=12

* $p \leq 0.05$ versus Control-operated animals

** $p \leq 0.01$ versus Control-operated animals

*** $p \leq 0.0001$ versus Control-operated animals.

$p \leq 0.05$ versus sham-operated animals.

$p \leq 0.0001$ versus sham-operated animals

CO (control), Sham, N (DECA), S (swimming) and NS (DECA+ swimming); and group B: Sham M (sham melatonin), M (melatonin), MN (melatonin +DECA), MS (melatonin+swimming), MNS (melatonin, DECA+swimming)

Discussion

Reduction in the body weight change was observed in the N, S and specially NS groups (Table1). Both exercise and AAS are muscle made and fat expenditure which affect body weight changes. On the other side, repair was seen in treated groups with melatonin by removing the oxidative stress from organs, and it was so prominent in MS and MNS groups (8.62 ± 2.48 , 4.53 ± 0.84 ; respectively) in compare with S and NS groups (4.53 ± 4.69 , 2.73 ± 2.3 ; respectively). Karakafi *et al.*, showed melatonin had no effect body weight in Mongolian Gerbils (24). Nevertheless, some studies mentioned that melatonin was able to affect energy intake and expenditure and showed a reduction in body weight (25).

Testes weight reduced in N, S and spatially in NS groups which were the same with the other reports (2,17,18,20). It is obviously known that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland have developmental effects on the testis. However, the administration of exogenous androgens such as AAS suppresses the serum LH and FSH level which can in turn has negative feedback effects on the H-P-G axis and results in suppression of endogenous testosterone (1).

Consequently, testicular atrophy occurs that resembles with our data. Moreover, it has been observed that exercise decrease the blood flow to the testes and causes low level of testosterone secretion which affects some degree of spermatogenesis (20), which closely resembles the diminution testes weight observed in the combination of exercise training and nandrolone decanoate treatment (NS group). We could see improvement of testes weight in melatonin treated groups (Table 1, Figures 6, 7) which were effective in MN, MS and specially in MNS groups. Ilbey *et al.*, showed melatonin could improve the cyclophosphamide and cisplatin effects on both body and testes weight by reduction of oxidative stress (26). Some studies show that swimming exercise can depress melatonin content in the rat pineal gland (6). It showed that exogenous melatonin as an antioxidant could improve the oxidative stress from swimming, DECA and swimming plus DECA.

In N, S and NS groups sperm parameters were affected. Decreasing of count, total and progressive motility and viability of sperms with increasing abnormality of sperms has been seen. Resemble Karbalay-Doust studies sperm quality was significantly defected by DECA in our study(27). Pey *et al.*, indicated that endurance exercise can result in dysfunction of the

male reproductive system (28,29). Severe azoospermia and oligospermia were also accounted in those who were given supraphysiological doses of anabolic-androgenic steroids (20). DECA and exercise are known to have destructive effects on sperm quality when constantly administered to rats (1).

In the present study, we showed that exposure to melatonin treatment for eight weeks caused the improvement of sperm progressive and total motility. In addition, based on our results, we observed better sperm viability and morphology in melatonin-treated groups.

The protective effect of melatonin on the oxidative changes in testis tissue and sperm characteristics was documented (29). Improvement of sperm quality is one of the earliest signs of the effects of melatonin on testicular function (30). Some studies showed Significant increases in the percentage of motility and percentage of progressive motility occurred when the spermatozoa were incubated with melatonin (24). Sarabia et al showed the protective effect of melatonin on damage in the sperm count and morphology of mice exposed to diazinon (31). According to Balao Da Silva et al., (32) and Casao et al., melatonin did not significantly impair motility characteristics. Casao et al., showed; melatonin treatment did not apply an effect on sperm viability (17,33). Although studies did not show any relation between sperm count and melatonin (34).

The valuable effect of melatonin at the mitochondrial level has been observed, which can include stimulatory effects on ATP construction (35). These protective effects of melatonin possibly related to the stimulatory effect of ATP creation.

The results of the study proved that the combined use of the two stimulants DECA and swimming can sever damage on sperm quality parameters, weight of testes. However, the administration of melatonin may improve some defects induced by swimming, DECA and swimming plus DECA.

This study concluded that melatonin probably could improve the sperm motility and sex organs weight after the combination of DECA and exercise. It probably shed some light in the improving male infertility in athletes that abuse AAS.

Acknowledgment

This study was financially supported by the Research Council of Tehran University of Medical Sciences, Iran.

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