Journal of Pharmaceutical Research International

32(14): 54-63, 2020; Article no.JPRI.59588
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

The Effect of Propranolol on Sperm Parameters, CatSper 2 Gene and Protein Expression, and Oxidative Stress in Adult Mice

Shabnam Mohammadi[1*], Farimah Beheshti[2], Sepideh Elyasi[3] and Mehdi Jalali[1]

[1]Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[2]Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran.
[3]Department of Clinical Pharmacy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Authors' contributions

This work was carried out in collaboration among all authors. Author SM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FB and SE managed the analyses of the study. Author MJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1430606
Editor(s):
(1) Dr. N. Alyautdin Renad, Scientific Centre for Expert Evaluation of Medicinal Products, Moscow, Russia.
Reviewers:
(1) Geovane Aparecido Ramos da Silva, Maringá State University, Brazil.
(2) Omer Faruk Ozkan, Turkey.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59588

Original Research Article

Received 24 May 2020
Accepted 29 July 2020
Published 08 August 2020

ABSTRACT

The aim of this study was to investigate the effects of propranolol on sperms, histopathology of testes, and *CatSper* 2 gene and protein expression in adult mice. 18 adult male mice were randomly divided into control, propranolol 1 (receiving 10 mg/kg dose) and propranolol 2 group (receiving 15 mg/kg dose for 35 days). The mean amount of sperm parameters in the propranolol 1 and propranolol 2 groups was significantly lower than the control group ($p<0.05$). *CatSper*2 gene and protein expression have significantly decreased in propranolol 1 and propranolol 2 groups compared to the control group ($P<0.05$). Reduction of *CatSper*2 gene and protein expression in low dose of propranolol was more severe than high dose. In testicular tissues of the propranolol 1 group, vacuoles and necrosis in the germinal epithelium were observed, and in testicular tissues of

**Corresponding author: E-mail: mohammadish@mums.ac.ir, shabnammhmmd@yahoo.com;*

propranolol 2 group decrease in the thickness of the germinal epithelium, some vacuoles and necrosis were observed in germinal epithelium as well as congestion in the interstitial space. The mean value of thiol and catalase enzyme in the propranolol 1 and propranolol 2 groups, and the mean value of superoxide dismutase in propranolol 1 group, were significantly different compared to the control group (P<0.05).

Keywords: CatSper; mice; sperm; testis; gene; protein; propranolol.

1. INTRODUCTION

According to World's Health Organization reports, cardiovascular diseases (CVD) are among the most important causes of mortality in the world. In recent years, cardiovascular diseases, including chronic pulmonary disease and cancer, have been the cause of patients' hospitalization in health center and hospitals. The cost of hospitalization for cardiovascular disease only in 2008 was more than 297 billion $. Thousands of people daily refer to hospitals and health centers with cardiovascular disease. In Asia, the prevalence of cardiovascular diseases is increasing and it costs a lot [1]. Beta-blockers are major therapeutic drugs for cardiovascular patients, especially in patients with heart failure. Beta-blockers are divided into two general categories of selective beta-blocker, such as esmolol, atenolol and metoprolol, and non-selective beta-blockers such as carvedilol, timolol, and propranolol .

Propranolol inhibits the connection of sympathetic neorucepters and thus reduces cardiac output, by blocking beta adrenergic receptors. In addition, causes vasodilatation in arteries and reduces their environmental resistance [1]. Propranolol brands are Inderal and Peranol, which are components of beta-1 and beta-2 receptor blocker drugs prescribed in pills for treating arrhythmia or blood pressure [2]. The side effects of their consumption are drowsiness, blurred vision, weakness and fatigue, dizziness, difficulty in defecation, sleep problems and decreased sexual ability [2].

Some studies investigate the adverse effect of beta-blocker drugs on motility of sperm, acrosome reaction and sperm capacity. Gavaging 15 mg/kg of propranolol for 60 days caused a significant reduction in sperm motility in rats. Also, degeneration in testicular tissues, epididymis and, seminal vesicle were observed. 7.5 mg/kg and 15 mg/kg doses of propranolol, have increased sperm with abnormal morphology and have decreased testosterone levels [3].

Gavaging 10 mg/kg and 15 mg/kg of propranolol, have reduced body and reproductive organs weights in rats. In addition, a significant decrease was observed in the population of primary and secondary spermatocyte cells and spermatid as well as sperm concentration and motility. At high doses of propranolol, FSH and LH levels of testosterone, were decreased compared to the control group. The number of absorbed embryos was also increased in groups that received 15 mg/kg of propranolol [4].

The in-vitro administration of propranolol have significantly reduced sperm motility [5-7] and it had adverse effects on sperm capacity, acrosome reaction, and sperm penetration in oocyte [8]. On the other hand, no study has been conducted about the effect of propranolol on CatSper gene and protein expression. The CatSper gene family contains CatSper 1-4 and beta and gamma [9]. CatSper genes are the key genes in male fertility expressed exclusively in the testes [10]. Hence, the aim of this study was to investigate the effects of propranolol on sperm parameters, histopathology of testes CatSper2 gene and protein effusion in adult male mice.

2. MATERIALS AND METHODS

18 adult male mice were kept in standard conditions (room temperature of 22 ± 2 °c, content moisture of 50 ± 5%, 12 hours of darkness and 12 hours of lightness) and randomly divided into 3 groups: Control, Propranolol 1 (receiving 10 mg/kg dose) and Propranolol 2 (receiving 15 mg/kg dose) [4].

Administration was applied by gavaging, for 35 days. The experiment was carried out for 35 days, and after deep anesthesia of mice with ether, sperm analysis was used. Left testicular tissue was sampled for molecular evaluation and the right testes was sampled for histopathological and biochemical studies.

2.1 Sperm Analysis

After deep anesthesia, epididymis from the inferior part of the mice abdomen was removed

and put inside a plate containing PBS and placed in CO_2 incubator for 30 minutes. For counting, 10 µ sperm solution was given and counted in 5 microscopic fields using 40 lens. Head, neck and tail morphology of sperm were also investigated. To evaluate sperm motility, the motile sperm was counted, and expressed as percentages in 5 fields [11].

2.2 Real Time – PCR

CatSper gene expression was determined using the Real time- PCR method. Initially, the extraction of RNA from tissues was carried out according to Parstous kit protocol. Then, the complementary DNA was prepared by Parstous cDNA Kit. The relative expression of *CatSper* was investigated by using sybr green, primers and Actin-β. Pfaffi et al's., methods were used for analyzing data [12].

2.3 Western Blot

After protein extraction and determining its value using Bradford method, the protein bands were transferred to gel electrophoresis. Then, the proteins were transferred to the nitrocellulose membrane. After blocking the nitrocellulose membrane, proteins were incubated with primary antibody (mouse anti-Rabbit *CatSper2* antibody dilution 1:100; Biorbyt, UK) and after that incubation was washed with secondary antibody (1:1000 dilution; HRP-conjugated goat anti-rabbit IgG; Abcam, USA). Protein was normalized with beta-actin, after using quantitative method for determining [13].

2.4 Histopathological Study of Testicular Tissue

Right testicular tissues was placed in the 10% formalin solution for 72 hours. Then, dehydration was performed using ascending alcohol (50, 60, 70, 80, 90 and 100%) and the clearing was carried out with two container of xylene. After infiltrating with paraffin in 60^{oC}, the molds were sectioned using rotary microtome. Hematoxylin-Eosin staining (Merck, Germany) was used for examining the samples and was investigated with optical microscopy [14].

2.5 The level of Malondialdehyde

1 ml of homogenized tissues were mixed with trichloroacetic acid and placed in water bath for 45 minutes. After cooling, it was centrifuged with 1000 rpm for 10 minutes and its absorption was read in 532 nm using a spectrophotometer, and the concentration of Malondialdehyde was calculated in moll/g [15].

$$\text{MDA concentration} = \text{absorbance}/1.56 \times 10^5$$

2.6 Measuring the Level of Thiol in Groups

The buffer was added to 50 µL of homogenized tissues, and adsorption was read at 412 nm by the spectrophotometer (A_1). Then, 20 µL of dinitrobenzoic acid was added and after 15 minutes incubation at room temperature, absorption was read at 412 nm (A_2). The adsorption rate of blank was considered as B and the following formula was used for calculating the amount of thiol concentration in µmoll/g [15].

$$(A_2\text{-}A_1\text{-}B)\times 1.07/0.05\times 13.6$$

2.7 Determining the Level of Catalase Enzyme

30 moll of hydrogen peroxide was added to the homogenized tissues of the sample placed in sodium phosphate buffer 50 mmoll. Then, the sample was placed in a spectrophotometer and optical absorption was performed at the wavelength of 240 nm [16].

2.8 The Level of Superoxide Dismutase

This enzyme was measured using Madesh method. After placing homogenized tissues in 10 mm of phosphate buffered saline with PH = 7.4, standard enzyme solution was prepared with different concentrations, and standard curves were drawed. Then, 65 µL of phosphate buffered saline, 30 µL (MTT 1.25 mmoll), and 75 µL of pyrogallel (100 µmoll) were mixed with 10 µL of tissues, and incubated for several minutes at room temperature. After that, 75 µL dimethyl sulfoxide were added and absorption was read at 570 nm wavelength, using ELISA reader [17].

2.9 Statistical Methods

Data were analyzed using SPSS software, ANOVA and Tukey test.

3. RESULTS

3.1 The Results of Analyzing Sperm Concentration, Motility and Morphology

The mean number of sperm in the control group was 4.60 ± 0.32, whereas after receiving 10 mg/kg of propranolol sperm concentration was 3.91 ± 0.20, which was significantly decreased compared to the control group (p = 0.001). The sperm concentration in the group receiving 20 mg/kg of propranolol (p = 0.001) was significantly decreased compared to the control group (Table 1). The mean of sperm motility in the control group was 81.12 ± 8.65 and it was 71.12 ± 7.51 after receiving 10 mg/kg of propranolol and decreased significantly (P=0.021). Statistical analysis showed a significant difference between sperm motility in propranolol 2 group (p=0.001) and the control group. Administrating 10 mg/kg of propranolol caused a significant decrease in the normal morphology rate of sperm compared to the control group (p=0.001) which was 88.12 ± 8.65 in the control group and 76.25 ± 3.95 in the propranolol 1 group (p = 0.001). The administration of propranolol 2 group led to a significant decrease in the normal morphology rate of sperm compared to the control group (p = 0.002).

3.2 The Results of Investigating *Catsper*2 Gene Expression in Testicular Tissues

CatSper 2 gene expression was investigated by Real-Time-PCR method in mice. As you see in Fig. 1, the expression of *CatSper* 2 gene was 0.70 ± 0.14 mg/kg in the propranolol 1 group, and 0.9 ± 0.14 in the propranolol 2 group. The results of statistical analysis showed that the gene expression in the propranolol 1 group (P = 0.001) and propranolol 2 group (p = 0.31) was significantly decrease compared to the control group. Statistically, there was no significant difference between CatSper2 gene expression in propranolol 1 and propranolol 2 groups (p = 0.02).

3.3 The Results of Investigating *Catsper*2 Protein Expression in Testicular Tissues

CatSper 2 protein effusion was investigated in mice, using Western blot method, after propranolol administration (Fig. 2). *CatSper* 2 protein effusion was significantly decreased in propranolol 1 group compared to the control group (P< 0.01). *CatSper* 2 protein effusion was significantly decreased in propranolol 2 group compared to the control group (P<0.01). The reduction of *CatSper* 2 protein expression in high doses of propranolol was more severe than low dose.

3.4 Histopathological Study on Testicular Tissues

In microscopic observations, the seminiferous tubules in the control group were with normal appearance, thickness, and interstitial tissues. Seminiferous tubules were containing sperm (Fig. 3). In testicular tissues in the propranolol 1 group vacuoles and necrosis in the germinal epithelium were observed and in testicular tissues in propranolol 2 group decrease in the thickness of the germinal epithelium, some vacuoles, and necrosis and degeneration in germinal epithelium were observed, as well and blood vessels congestion in the interstitial space (Fig. 3).

3.5 The Results of Analyzing the Level of Malondialdehyde (MDA)

Fig. 4 shows the value of malondialdehyde in testicular tissues in different groups in nmol/mg. The mean malondialdehyde level in the control group was 3.21 ± 0.43 nmol/mg, while in propranolol 1 group it was 3.31 ± 0.68. The statistical analysis did not show a significant difference between the mean malondialdehyde level in propranolol 1 group and the control group (p = 0.96). The mean malondialdehyde level

Table 1. The effect of propranolol administration on sperm concentration, motility and morphology of sperm in different groups

Group	Sperm concentration (million/ml)	Sperm motility (%)	Percent of sperm with normal morphology (%)
Control	4.60 ± 0.32	81.12 ± 8.65	88.12 ± 8.65
Propranolol 1	3.91 ± 0.20*	71.12 ± 7.51*	76.25 ± 3.95*
Propranolol 2	3.63 ± 0.26*	66.12 ± 3.04*	78.00 ± 3.54*

** P<0.05 was significantly different with the control group*

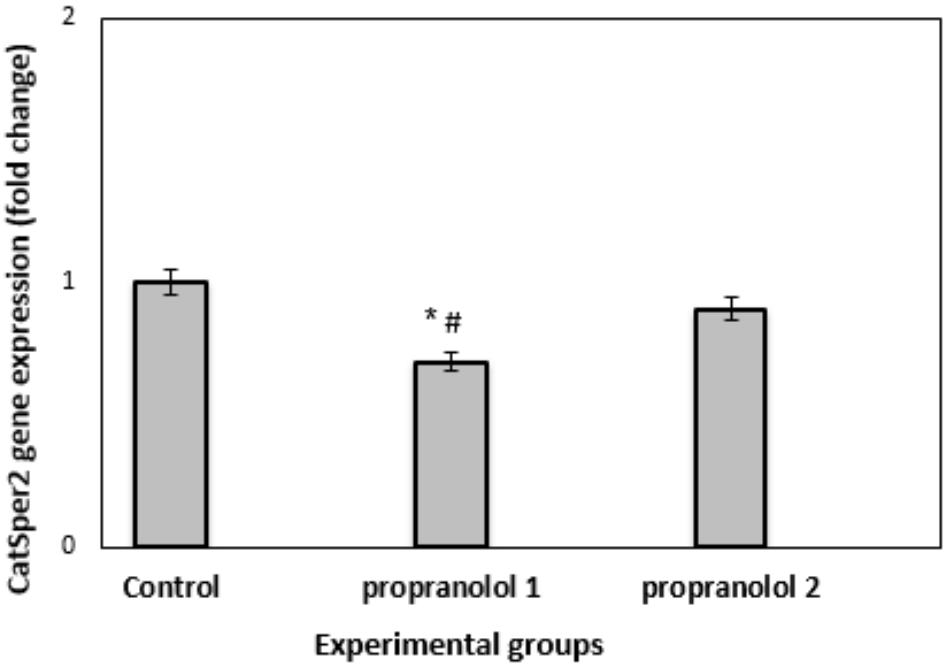

Fig. 1. Expression of *CatSper2* gene in testicular tissues in different groups

**P=0.001 was significantly different with the control group*
 # P=0.02 was significantly different with the propranolol 2 group

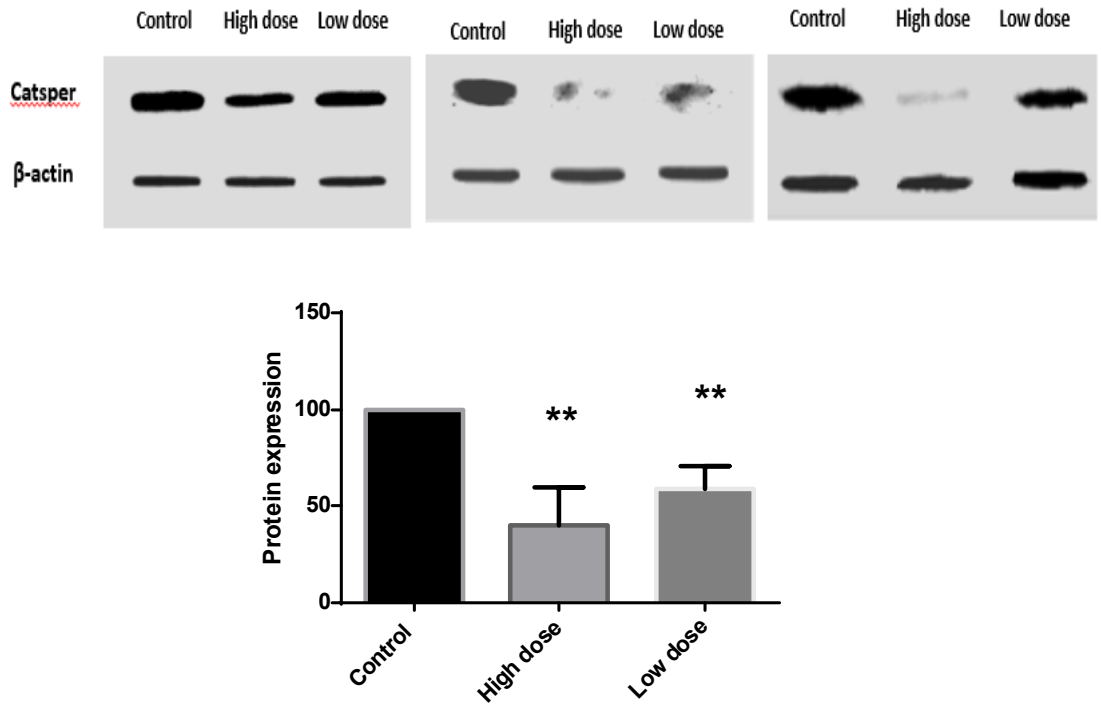

Fig. 2. The expression of *CatSper* 2 protein in testicular tissues in different groups

***p <0.01 was significantly different with the control group*

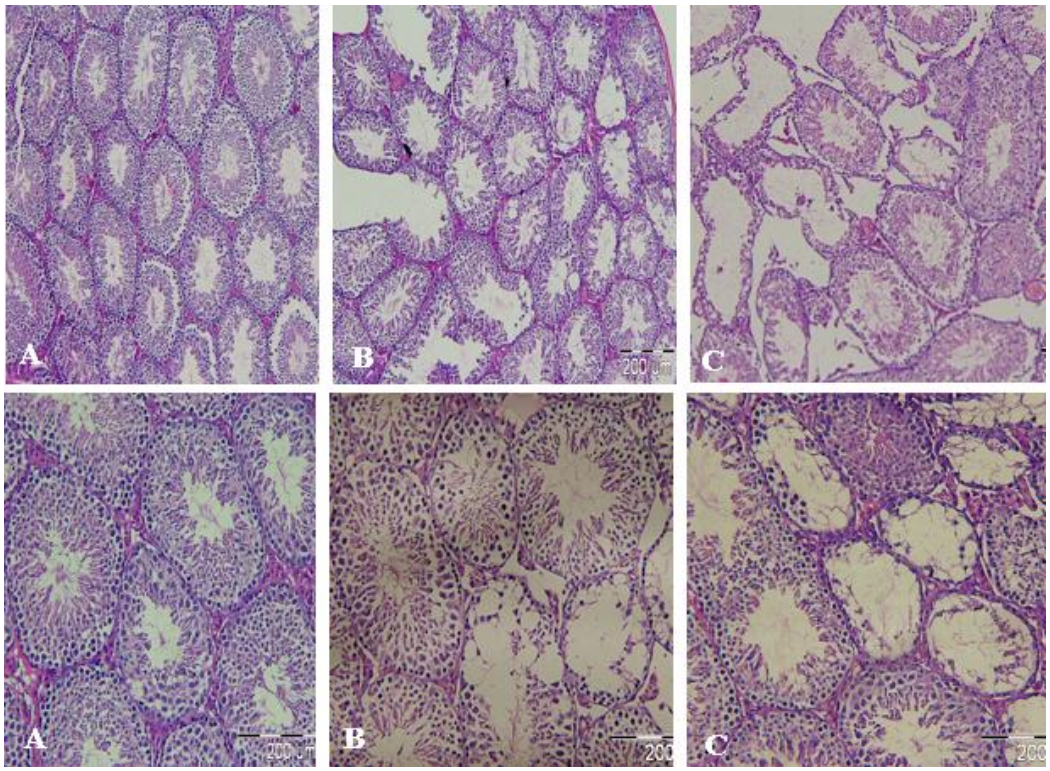

Fig. 3. Transverse section of testis of the mice in the control (A), propranolol 1 (B), and propranolol 2 (C) groups (H & E staining)

in the control group was 3.21 ± 0.43 nmol/mg, while in propranolol 2 group it was 2.17 ± 1.01. No significant difference was observed in propranolol 2 group compared to the control group (p = 0.08).

3.6 The Results of Analyzing the Level of Thiol

Fig. 4 shows the values of thiol in testicular tissues in different groups, in µmol/g. The mean value of thiol in the control group was 0.56 ± 0.10 nmol/g, while in propranolol 1 group, it was 0.23 ± 1 0.07 and in propranolol 2 group, it was0.34 ± 0.02. The statistical analysis showed a significant difference between the mean value of thiol level in propranolol 1 (p = 0.001) and propranolol 2 groups (p = 0.001) compared to the control group.

3.7 The Results of Analyzing Catalase Enzyme

Fig. 5 shows the amount of catalase enzyme in different groups (U/g). The mean value of catalase enzyme level in the control group was 0.99 ± 0.12 U/g, while it was 0.50 ± 0.01 in the propranolol 1 group and 0.07 ± 0.01 in propranolol 2 group. The statistical analysis showed a significant decrease in the mean level of catalase enzyme in propranolol 1 (p = 0.001) and propranolol 2 (p = 0.01) groups compared to the control group.

3.8 The Results of Analyzing Superoxide Dismutase Enzyme

Fig. 6 shows the amount of superoxide dismutase enzyme in different experimental groups in U/g. The mean level of superoxide dismutase enzyme in the control group was 12.81 ± 3.14 U/g, while it was 1.72 ± 0.47 in propranolol 1 group and 10.88 ± 2.37 in propranolol 2 group. The statistical analysis showed a significant decrease in the mean level of superoxide dismutase in the propranolol 1 group (p = 0.001) compared to the control group. There was no significant difference in the mean level of superoxide dismutase in propranolol 2 group (p = 0.36) compared to the control group.

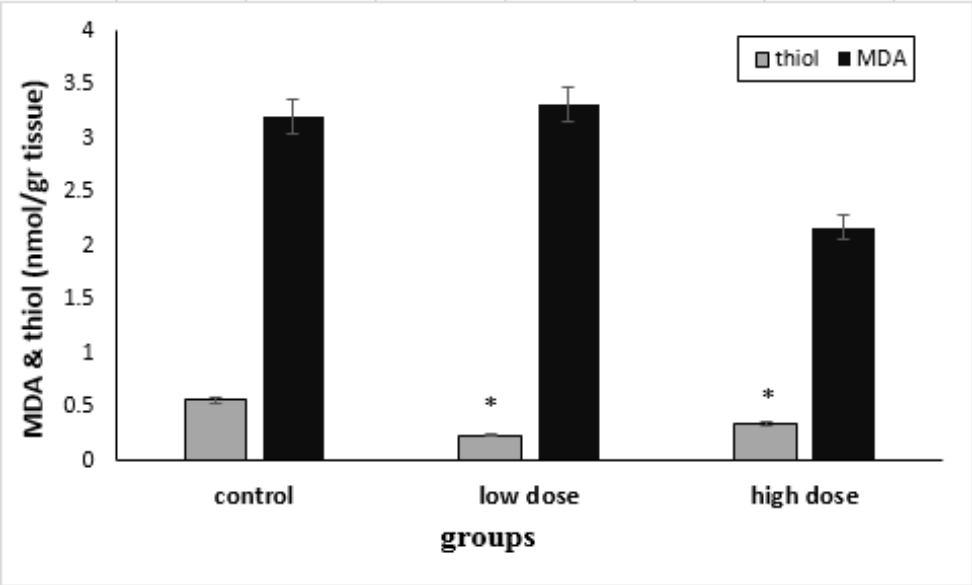

Fig. 4. Mean value of malondialdehyde and thiol levels in testicular tissues in different groups
**P<0.05 was significantly different with the control group*

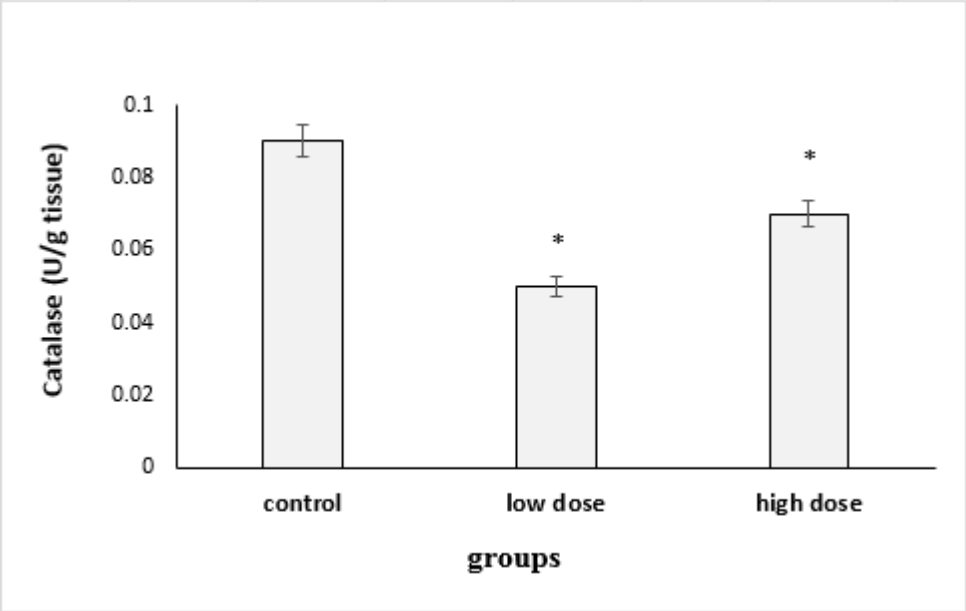

Fig. 5. The mean value of catalase level in testicular tissues in different groups
**P<0.05 was significantly different with the control group*

4. DISCUSSION

In the present study, 10 mg/kg and 15 mg/kg of propranolol were administered to mice in by gavaging [4]. The length of the administration period was selected according to the length of Spermatogenesis in mice which is 35 days [18]. The results showed that the mean value of sperm parameters in the propranolol 1 and propranolol 2 group were significantly decreased compared to the control group. In testicular tissues in the propranolol 1 group vacuoles and necrosis in the germinal epithelium were observed and in testicular tissues in propranolol 2 group decrease in the thickness of the germinal epithelium, some vacuoles, and necrosis and

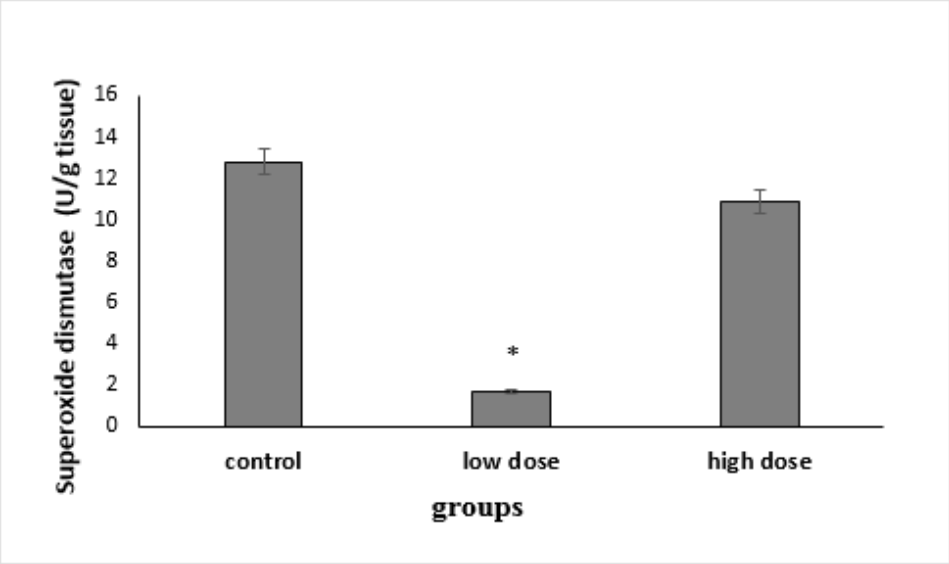

Fig. 6. The mean value of superoxide dismutase level in testicular tissues in different groups was significantly different with the control group P<0.05 *

degeneration in germinal epithelium were observed, as well and blood vessels congestion in the interstitial space. According to our results, the investigation of the effects of atenolol, metoprolol, and propranolol drugs on sperm quality, and histopathology of testicular in rats for 60 days, showed that administrating 9 and 18 mg/kg of atenolol, 3.5 and 7 mg/kg metoprolol and 15 mg/kg propranolol, caused a significant reduction in sperm motility. In addition, degeneration in testicular tissues, epididymis and seminal vesicles were observed. Administrating high doses of atenolol and metoprolol, and 7.5 and 15 mg/kg of propranolol, increased sperm with abnormal morphology. Atenolol administration caused no significant changes in testicular, epididymis, and seminal vesicles weights. In all groups, the beta blocker drugs were used to decrease testosterone levels at the end of the experiment. The evaluation of testosterone levels in 90 days after the onset of the experiment, also showed a reduction, but it returned 60 days after the drug discontinuation [3].

Another study in 2007 investigated the effects of two doses of 10 and 15 mg/kg of propranolol on mouse genital system. It was administered to adult male mice by gavaging for 35 days. The results showed that the body, testicular, epididymis, prostate and the seminal vesicle weight decreased in the groups receiving drugs. In addition, a significant decrease was observed in the population of primary and secondary spermatocyte cells and spermatid as well as sperm count and motility. At high doses of propranolol levels of testosterone, FSH and LH were decreased compared to the control group. The number of absorbed embryos was also increased in the group that had received 15 mg/kg propranolol [4].

In another study, it was reported that, the sperm motility was significantly decreased, by administrating beta blockers such as trazodone, nadolol, and propranolol on human sperm in the in-vitro.

Another study reported that damages of penbutolol and propranolol on sperm are greater than alprenolol, oxprenolol and metoprolol [6].

In our study, it was indicated that propranolol has an adverse effect on sperm parameters. No study has been found on the effect of beta blocker drugs on *CatSper* gene and protein expression. However, studies show that propranolol has devastating effects on sperm capacity, acrosome reaction and sperm penetration to zona pellucida [18,5].

On the other hand, sperm requires hyperactivated motility to pass through egg coatings. It seems that hyperactivity with flow calcium ions is caused by the *CatSper* channel. Studies show that, the elimination of *CatSper*1

gene causes impairment in sperm motility, its penetration to oocyte and calcium ion, and sterility in mice [19]. The elimination of *CatSper*2 gene leads to a complete sterility of male mice and impaired hyperactivated motility, sperm capacity and penetrating to zona pellucida [20]. *CatSper*3, 4 are mainly in the acrosome area of sperm head and have been observed to play an important role in the acrosome reaction [21].

Propranolol decreases CatSper 2 protein expression via reduction calcium flow in sperm cells. By considering that the beta-adrenergic receptor are on both the head and tail of the sperm, this mechanism may be that propranolol connection to this receptors causes the changes in calcium flow in the sperm calcium channels, especially on unique calcium channel of *CatSper* [22]. Another mechanism is that the reduction of sperm motility in mice receiving propranolol due to the oxidative stress, caused by reducing the amount of axonemal phosphorylation of sperm tail and damages to the sperm flagellum, reduces the rate of sperm motility or may cause oxidative stress to the sperm mitochondria as an important cytosolic organ. In this study, the effects of propranolol administration on *CatSper* gene and protein expression was investigated for the first time. However, it was better to evaluate the acrosome reaction and calcium flow in calcium channels. The limitation of this study was lack of financial budget. It was better to investigate all *CatSper* family genes and proteins instead of one gene and protein. Evaluating propranolol administration in different times and dosages is recommended for researchers.

5. CONCLUSION

The results of this study showed that propranolol, in a dose-dependent manner, can cause testicular histopathology damage, and increase oxidative stress. In addition, it reduces sperm parameters and *CatSper* 2 gene and protein expression.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This experimental study was approved by the Ethics Committee (IR.MUMS.MEDICAL.REC. 1397.616) Mashhad University of Medical Sciences.

ACKNOWLEDGEMENT

This article is extracted from a research project (code 970989) of Mashhad University of Medical Sciences, which is hereby thanked and appreciated by the aid. He also thanked and appreciated Mr. Feizi and Ms. Amamian in central laboratory would also be thanked. In addition, Dr. Farzad Rahmani and Mr. Mohammad Jalili Nik in biochemistry department would be thanked.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ivor Benjamin, Robert C. Griggs, Edward J Wing, Gregory Fitz J. Andreoli and Carpenter's Cecil Essentials of Medicine, 9th Edition. Translated by Yaghobi B, Shabani H, Tari k, Shamsi Z, Nayebi R, Aminmanbari A, Mosavi M, Ebrahimi L, Mohammadiha A. 2016;1399-1474.
2. Al-Majed AA, Bakheit AHH, Abdel Aziz HA, Alajmi FM, AlRabiah H. Propranolol. Profiles Drug Subst Excip Relat Methodol. 2017;42:287-338.
DOI: 10.1016/bs.podrm. 2017.02.006
3. El-Sayed MG, el-Sayed MT, Elazab Abd el S, Hafeiz MH, el-Komy AA, Hassan E. Effects of some beta-adrenergic blockers on male fertility parameters in rats. Dtsch Tierarztl Wochenschr. 1998;105(1):10-2.
4. Nusier MK, Bataineh HN, Daradka HM. Adverse effects of propranolol on reproductive function in adult male mice. Pak J Biol Sci. 2007;10(16):2728-31.
5. Semet M, Paci M, Saïas-Magnan J, Metzler-Guillemain C, Boissier R, Lejeune H, Perrin J. The impact of drugs on male fertility: A review. Andrology. 2017;5(4): 640-663.
6. Curtis-Prior PB, Gadd AL. Beta-adrenoceptor antagonists and human sperm motility. J Pharm Pharmacol. 1990; 42(3):220-2.
7. Shaliutina O, Shaliutina-Kolešová A, Lebeda I, Rodina M, Gazo I. The *in vitro* effect of nonylphenol, propranolol, and diethylstilbestrol on quality parameters and oxidative stress in sterlet (*Acipenser ruthenus*) spermatozoa. Toxicol *in vitro*. 2017;43:9-15.
DOI: 10.1016/j.tiv.2017.05.006.

8. Zhong CL, Ge RS, Shi QX. Inhibition of guinea pig sperm capacitation, the acrosome reaction and sperm-egg fusion by dl-propranolol *in vitro.* Zhongguo Yao Li Xue Bao. 1990;11(2):169-72.
9. Rahban R, Nef S. CatSper: The complex main gate of calcium entry in mammalian spermatozoa. Mol Cell Endocrinol. 2020; 110951.
DOI: 10.1016/j.mce.2020.110951
10. Finkelstein M, Etkovitz N, Breitbart H. Ca2+ signaling in mammalian spermatozoa. Mol Cell Endocrinol. 2020; 110953.
DOI: 10.1016/j.mce.2020.110953
11. Mohammadi, S, Gholamin, M, Mohammadi M, Mansouri A, Mahmoodian R, Attari S, …, Ferns, GAA. Down-regulation of CatSper 1 and CatSper 2 genes by lead and mercury. Environ Toxicol Pharmacol, 2018;59:82-86.
DOI: 10.1016/j.etap.2018.03.007
12. Mohammadi S, Gholamin M, Mansouri A, Mahmoodian R, Babazadeh B, Kebriaei SM, Zibaei B, Roshanaei M, Daneshvar F, Khandehro M, Khodadadegan MA, Delshad A, Mohammadzadeh F, Mohammadi M, Sadeghi S , Shoeibi S, Boroumand-Noughabi S, Ghayour-Mobarhan M, Tavallaie S, Vafaei A, Ferns GAA. Down-regulation of CatSper 2 gene by cadmium and nickel. Toxin Reviews. 2018;37:3,216-222.
13. Mohammadi S, Rahmani F, Hasanian SM, Beheshti F, Akbari Oryani M, Ebrahimzadeh A, Farzadfar S. Effects of dioxin on testicular histopathology, sperm parameters, and CatSper2 gene and protein expression in Naval Medical Research Institute Male Mice. Andrologia. 2019;00:e13411.
14. Attari S, Mohammadi S, Ebrahimzadeh A, Hosseinzadeh H, Soukhtanloo M, Rajabzadeh A. effects of thymoquinone on sperm parameters, apoptosis, testosterone level, and oxidative stress in a mouse model of D-galactose-induced aging. Pharm Sci. 2018;24(3):180-186.
15. Adikwu E, Bokolo B. Prospects of N-Acetylcysteine and melatonin as treatments for tramadol-induced renal toxicity in albino rats. Pharm Sci. 2017; 23(3):172-81.
16. Kalantar M, Shirali S, Hasanvand A, Valizadeh M, Tavakoli R, Asadi M, et al. Ameliorative effects of hydroalcoholic extract of *Lavandula officinalis* L. on methotrexate-induced oxidative stress in rats. Pharm Sci. 2017;23(1):18-26.
17. Vafaei A, Mohammadi S, Fazel A, et al. Effects of Carob (*Ceratonia siliqua*) on sperm quality, testicular structure, testosterone level and oxidative stress in busulfan-induced infertile mice. Pharm Sci. 2018;24(2);104-111.
18. Chan SY, Tang LC, Chan PH. Effect of propranolol on human spermatozoal *in vitro* hamster ova penetration. Arch Androl. 1984;12:79-82.
19. Eswari Beeram, Bukke Suman, Bysani Divya. Proteins as the molecular markers of male fertility. J Hum Reprod Sci. 2019; 12(1):19-23.
DOI: 10.4103/jhrs.JHRS_9_18
20. Lin Yang, Hai-Xia Chen, Xiao-Huan Mu, Xiao-Qiang Liu, Xue-Ru Song , Wen-Yan Tian . CatSper in sperm hyperactivation and male infertility: Advances in studies. Zhonghua Nan Ke Xue. 2017;23(8):751-756.
21. Xiang-Hong Sun, Ying-Ying Zhu, Lin Wang, Hong-Ling Liu , Yong Ling, Zong-Li Li , Li-Bo Sun. The Catsper channel and its roles in male fertility: A systematic review. Reprod Biol Endocrinol . 2017;15(1):65.
DOI: 10.1186/s12958-017-0281-2.
22. Adeoya-Osiguwa SA1, Gibbons R, Fraser LR. Identification of functional alpha2- and beta-adrenergic receptors in mammalian spermatozoa. Hum Reprod. 2006;21(6): 1555-63.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59588