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# The effect of vitamin D treatment on the sperm parameters of mice treated with busulfan

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**Abstract**

Spermatogenesis is a very complex process that can be affected by a number of factors which may lead to reduced fertility or infertility. Busulfan kills spermatogonia stem cells and disrupts the connections between Sertoli and spermatogonia cells at base layers. Moreover, vitamin D is very important in the reproductive system of men. In this study, the effect of vitamin D on biosulfur-induced azoospermia in mice was investigated. The results showed an increase in the quality of sperm parameters in the group treated with vitamin D.

## INTRODUCTION

Infertility is one of the most common health problems in the world, affecting about 10% to 15% of couples and causing infertility in about 50% of men. More than half of all cases of infertility in men are due to dysfunction (1). Spermatogenesis is a very complex process that can be affected by a number of factors which may lead to reduced fertility or infertility (2). Chemotherapy and radiation therapy are associated with many changes in the male reproductive system. One drug used in treating cancer is busulfan, a white, crystalline powder with the scientific name of 1.4 butanediol-dimethane sulfonate. Busulfan is an alkylating agent and a cytotoxic drug belonging to the alkyl sulfonate group (3). It reacts with nucleophiles and proteins, causing a cross-link between DNA-DNA and DNA-Protein to damage DNA, and breaks down the bonds between guanine-guanine bases inside DNA. This group of drugs is used to treat chronic leukemia, ovarian cancer, and pre-bone marrow transplantation in cancer patients (4). Like other alkylating drugs, busulfan has carcinogenic and teratogenic properties. It kills spermatogonia stem cells and disrupts the connections between Sertoli and spermatogonia cells at base layers (5). Alkylating drugs lead to azoospermia in 90-100% of men and to ovarian dysfunction in 5-25% of women. Many recent studies have shown that in addition to regulating calcium homeostasis, bone mineralization, cell differentiation, and proliferation, vitamin D is also very important in the reproductive system of men (6). Numerous studies have shown that vitamin D receptors and their metabolizing enzymes are present in the reproductive system of males and in adult human spermatozoa (7). These findings suggest that vitamin D can have a wide range of positive biological effects on the health of the reproductive system by binding to and activating its receptors (8). The current study investigated the effect of vitamin D on biosulfur-induced azoospermia caused by treatment with busulfan in mice. Vitamin D used in the treatment of mice was expected to have positive effects on sperm parameters due to its antioxidant properties.

## MATERIALS AND METHODS

For this study, 20 NMRI mice with an average weight of 17.67 grams were purchased from the Pasteur Institute and transferred to the laboratory. The animals were kept in a limited number of cages in a special room for rats at 23 °C. Humidity was set at 45%, and a 12-hour dark/light period was observed. Water and common mouse food were freely available to the mice until the end of the protocol. The mice were divided

into four groups: the control group, the busulfan treatment group, the vitamin D treatment group, and the busulfan + vitamin D treatment group. Busulfan (Sigma, St. Louis, MO) was dissolved in DMSO 2% and sterile distilled water with a ratio of 1: 1 at room temperature. The control group subcutaneously received 100 µM DMSO (Sigma, St. Louis, MO) per day for 4 days, and the busulfan treatment group received 3.2 mg/kg busulfan intraperitoneally for 4 days. Vitamin D was prepared as a capsule; the solution inside the capsule was drained and administered in doses of 75 mg per kilogram of mouse weight with olive oil. Treatment with vitamin D was performed as a gavage for 20 days. After the treatment period, the mice were anesthetized under sterile conditions with ether. The epididymis was removed, immediately cut with sterile scissors, and placed in an isotonic solution of 4 ml of phosphate saline buffer. The temperature was set at 37 °C. At this point, the sperm fully exited the duct. The fully automatic device HFT-CASA was used to evaluate the sperm parameters.

## RESULTS

The results showed a decrease in the number of normal sperm, in sperm motility, and in survival in the busulfan group. Treatment with busulfan significantly reduced sperm count, but vitamin D was able to significantly increase sperm count compared to the control group. The use of busulfan also significantly reduced sperm motility, but vitamin D increased sperm motility significantly compared to the control group.

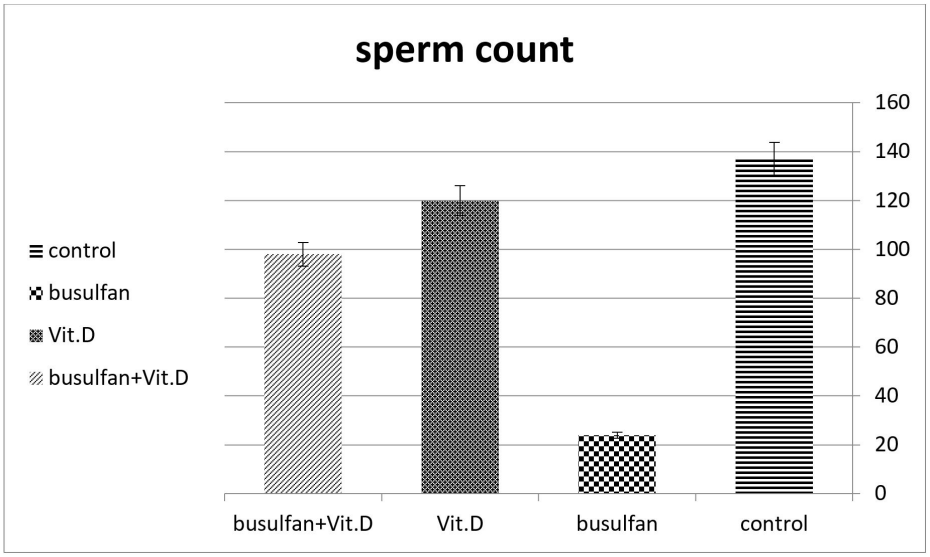


**Fig 1.** Effects of Vitamin D on the number of sperm in mice treated with bosulfan: Vitamin D significantly increases the number of sperm in mice treated with bosulfan

## DISCUSSION

The protective effects of vitamin D on spermatogenesis and sperm parameters in male mice treated with busulfan were investigated. Many recent studies have shown that in addition to regulating calcium homeostasis and bone mineralization, vitamin D plays an important role in the reproductive system (9). The findings have suggested that vitamin D can have a wide range of positive biological effects on the health of the reproductive system by binding to and activating the vitamin D receptor (VDR) (10). In humans, vitamin D receptors have been found in the seminiferous tubule of the testicles, prostate, nucleus, and sperm neck, indicating the prominent role of this vitamin in spermatogenesis and human sperm maturation (11). Studies have further shown that vitamin D deficiency or mutations in its receptor in mice reduce sperm count, reduce sperm motility, or cause testicular tissue abnormalities in the male reproductive system. The chemotherapeutic agent busulfan, an alkylating agent, is used to treat chronic leukemia and ovarian cancer and is administered before bone marrow transplantation in cancer patients (12). Azoospermia seeks to use busulfan as an important and significant complication that kills most spermatozoa. Busulfan affects spermatogonia germ cells by increasing the production of free radicals and the activity of oxidative stress (13). Suri et al. showed that vitamin D has antioxidant properties that can affect sperm quality (14). Other studies have shown that vitamin D increases the quality and quantity of sperm by increasing testosterone secretion. In own study also found that vitamin D plays a protective role against busulfan-induced azoospermia.

## REFERENCE

1. Allouche L, Hamadouche M, Touabti A. Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. Exp Toxicol Pathol. 2009 Sep; 61(5): 503-10.

2. Shan G, Tang T, Zhang X. The protective effect of ascorbic acid and thiamine supplementation against damage caused by lead in the testes of mice. J Huazhong Univ Sci Technolog Med Sci.

2009 Feb; 29(1): 68-72.
3. Graca A, Ramalho-Santos J, de Lourdes Pereira M. Effect of lead chloride on spermatogenesis and sperm parameter in mice. Asian J Androl. 2004 Sep; 6(3): 237-410.
4. Sokol RZ. The effect of duration of exposure on the expression of lead toxicity on the male reproductive axis. J Androl. 1990 Nov-Dec; 11(6):521-6
5. Anjum MR, Sainath SB, Suneetha Y, Reddy PS. Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. Ecotoxicol Environ Saf. 2011 May; 74(4): 793-9
6. Golshan- Iranpoor F, Emami E. The effects of lead on motility, viability and DNA denaturation of cauda epididymal spermatozoa of mouse. J Shahrekord Univ Med Sci. 2011Oct-Nov; 13(4):1-8. [full text in Persian]
7. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol. 2010; 7(1):351-385.
8. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008 Apr; 87(4):108-118.
9. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. J Biomed Biotechnol. 2012 Sep; 2012:634195.
10. Nimitphong H, Chanprasertyothin S, Jongjaroenprasert W, Ongphiphadhanakul B. The association between vitamin D status and circulating adiponectin independent of adiposity in subjects with abnormal glucose tolerance. Endocrine. 2009 Oct; 36(2):205-10.
11. Patel P, Poretsky L, Liao E. Lack of effect of subtherapeutic vitamin D treatment on glycemic and lipid parameters in Type 2 diabetes: Apilot prospective randomized trial. J diabetes. 2010 Mar; 2(1):36-40. 12- Foss YJ. Vitamin D deficiency is the cause of common obesity. Med Hypotheses. 2009 Mar; 7(2): 314–321.
12. Souri E, Farsam H, Hasani M, Azimi kheirabadi Z. Evaluation of antioxidant activity of 25 plant seeds used in Iranian folk medicine. JMP. 2003 Dec; 4(8):27-34.
13. Naziroğlu M. Enhanced testicular antioxidant capacity in streptozotocin-induced diabetic rats: protective role of vitamins C and E and selenium. Biol Trace Elem Res. 2003 Jul;94(1):61-72
14. Baghy Nia N, Oryan SH, Fani A, Maleky Rad A. The effect of Cardamom- tea watery extract on oxidative stress. AMUJ. 2008 Dec; 11(4):1-7. [full text in Persian]