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# Vitamin E improves the reproductive system of male rats exposed to busulfan chemotherapy

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**Abstract**

Vitamin E is a well-known antioxidant and is frequently used as an adjunct treatment in cancer therapy. Busulfan is a commonly used drug for cancer treatment. In this study, twenty-eight male rats, ten weeks old and weighing between 250 and 300 grams, were divided into four groups. The first group served as the control and received daily intraperitoneal injections of Dimethyl sulfoxide (DMSO) for four weeks. The second group received a single dose of Busulfan at 40 mg/kg body weight via intraperitoneal injection. The third group received the same single dose of Busulfan along with daily intraperitoneal injections of Vitamin E at 100 mg/kg body weight for four weeks. The fourth group was given only Vitamin E at the same dosage for four weeks. At the end of the experiment, the animals were sacrificed, and blood samples were collected to test antioxidant enzyme levels (GSH, SOD, CAT) and analyze serum concentrations of reproductive hormones (FSH, LH, and testosterone). Additionally, sperm motility and viability were assessed after collecting epididymal spermatozoa. The findings revealed that Busulfan significantly increased serum levels of both FSH and LH while causing a notable decrease in testosterone levels. Furthermore, Busulfan treatment resulted in a significant reduction in sperm count, motility, and viability, along with a marked increase in sperm morphological abnormalities. In contrast, supplementation with Vitamin E alongside Busulfan improved hormone levels and enhanced sperm function. In conclusion, Busulfan has a toxic effect on sperm and directly impacts body weight and testicular weight. However, Vitamin E demonstrates beneficial therapeutic effects on testicular tissue and enhances sperm production in rats treated with Busulfan.

**Keywords:** Busulfan, Male Reproductive System, Antioxidant

## 1. Introduction

Chemotherapy is the systematic use of anticancer drugs to treat cancer. This treatment targets the rapid growth and division of cancer cells. Regrettably, it lacks the selectivity to differentiate between normal and malignant cells. The toxic effects of chemotherapy are caused by the death of healthy cells although chemotherapy is an efficient way of treating many types of cancer, it is not without danger. There are both relatively harmless and potentially fatal adverse effects of chemotherapy [1].

Most chemotherapy medications target rapidly dividing cells by interfering with the process of mitosis (cell division). Alteration of DNA and blockage of cell-division processes are two ways to halt mitosis [2]. Blood-forming cells in the bone marrow, the hair follicle cells, and oral, digestive, and reproductive system cells are particularly susceptible to chemo's ill effects [3].

Busulfan is given to cancer patients before they undergo bone marrow transplantation, and it is used to treat chronic leukemia and ovarian cancer [4]. Busulfan is a powerful drug that preferentially destroys spermatogonial embryonic stem cells [5], unlike agents that destroy developed spermatogonia.

Vitamin E is a powerful antioxidant that can penetrate fat cells. This antioxidant is called "chain-breaking" because it can stop a chain reaction that free radicals start [6]. There is some evidence linking regular use of antioxidants with higher-quality semen in otherwise healthy males[7,8]. It has been found [9] that men with higher vitamin E intake also had more rapidly improving sperm motility and, in addition, taking a vitamin E supplement helped keep sperm DNA together [10]. Antioxidant defense systems, such as alpha-tocopherol (vitamin E), found in the seminal plasma and plasma membrane, often keep ROS levels to a minimum [11-13].

Fifty percent of infertility cases may be traced back to low-quality sperm from the male partner is likely underestimated due to data dependability problems caused by poor record-keeping and medical care.

The goal of the study was to determine the harmful effects of a single dosage of busulfan administered on the reproduction system of male rats (especially in the testis)

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and analyze the potential restorative effects of vitamin E on the mutated testicular cells brought on by busulfan administration.

## 2. Material and Methods

Busulfan's chemical name is 1,4-butanediol, dimethane-sulfonate. (IARC, (19982).

The main component is a white crystalline powder with a chemical formula of CH3SO2O(CH2)4OSO2CH3 and 250 g/mol. Busulfan 40 mg/kg was dissolved in 10 ml of dimethyl sulfoxide (DMSO; Wako, Japan) and diluted with 10 ml of sterile distilled water immediately before use (Fang, F., et al (2017).

White albino rats were chosen as the subject of the study model. The experimental animals used in this study were male *Rattus norvegicus* rats that were obtained from animal house in the College of Veterinary Medicine at the University of Kerbala in Iraq. Males were typically between (350) and (390) grams in weight and (2.5) to 3 months old. The creatures appeared to be in good health, having been raised in a clean, climate-controlled environment. From 12/10/2022 to 10/11/2022, the light system was 13± 2 hrs. light/dark cycle; 25± 2 c° with a relative humidity of 35 to 65%.

The animals were kept in cages made of plastic, and the drinking water was delivered in bottles made of glass. Daily, food and drink were provided. Before the beginning of the experiment, the animals were given one week to become accustomed to the laboratory conditions.

Twenty-eight male adult rats weighing between 320 and 380 grams were randomly split into four groups, each with seven rats in it. These groups were named after the following categories:

The first group, denoted by the letter "A," consisted of Seven male rats that were administered DMSO through intraperitoneal injection once daily (0.1 mL). These rats acted as the control.

The second group, known as Group B, received a single dose of busulfan (40 mg/kgIP.) through intraperitoneal injection [14]. This dose was administered to seven male rats.

A single dosage of busulfan (40 mg/kgIP.) and vitamin E (100 mg/kg, daily b.w/IP) was injected intraperitoneally into seven male rats in the third group, which was designated as Group C.

The fourth group, also known as Group D, consisted of six male rats given a dose of vitamin E through intraperitoneal injection (100 mg/kg, daily, body weight/IP)( Figure 1) [15]. After a period of 28 days during which the medications were delivered via intraperitoneal injection, the animals in each group would be sacrificed, and the animals that were (anesthetized by chloramphenicol inhalation) at the end of the trial. Thoracotomy and laparotomy were used to open the chest and abdomen. Following the collection of blood for biochemical and hormonal tests, caudal sperm was collected for caudal sperm analysis.

Ethical approval: ethics provides guidelines for the responsible conduct of experimental animals from Veterinary Medicine College University of Karbala.

Blood samples from each male rat were collected via heart puncture. The samples were placed in serum tubes and allowed to sit for 30 minutes. Using a 5 ml disposable syringe, blood was drawn directly from the heart and transferred to gel tubes. These tubes were then centrifuged

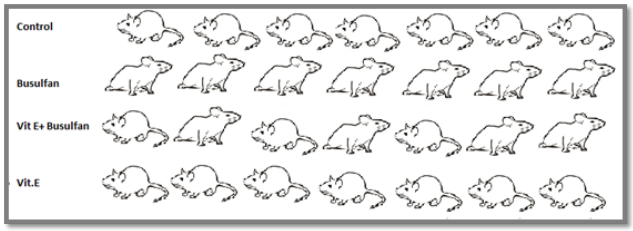


**Fig. 1.** Diaphragm represented experimental studies.

at 3000 rpm for 10 minutes and stored at -20 °C to separate the serum. The serum was subsequently transferred to 1.5 ml microcentrifuge tubes for analysis of reproductive hormone concentrations (FSH, LH, and testosterone). All tubes were kept at -4 °C until analysis. To minimize diurnal variation in hormone levels, all samples were collected in the morning between 9:00 AM and 12:30 PM.

Evaluation of serum concentration of the levels of the antioxidant glutathione (GSH). Measurements of glutathione, catalase, and superoxide dismutase were carried out in accordance with the methodology described in the research [16]. The number of motile sperm was estimated using the method described by (Lio, et al., 1986), and the Neubauer hemocytometer chamber used for RBC and WBC counts (Fernandes et al., 2011). Spermatozoa are collected for a test of sperm motility. The vitality of sperm was measured [17] as follows after epididymal spermatozoa were collected by cutting the caudal portion of the epididymis into tiny fragments in 2 ml of normal saline pre-warmed to 37°C.

### 2.1. Statistical analysis

The data was analyzed using SPSS version 25.00, and a one-way analysis of variance (ANOVA) was used to determine whether or not there were statistically significant differences between the groups. The LSD test was used to examine statistical significance between treatment means, and results were considered significant if the P value was less than 0.05 or greater than 0.05.

## 3. Results

The influence of Buselfan and Vitamin E on both the total body weight and the weight of the testicles; The findings presented in Table 1 are for the group of male rats that were given Busulfan, Busulfan in combination with Vitamin E, or Vitamin E by itself, for 28 days. The rats showed a significant ($p < 0.05$) decrease in body weight and testes weight in the Busulfan group and the Busulfan + Vitamin E group compared to the control group and the Vit E group (Figure 2).

### 3.1. The Buselfan and Vitamin E's Impact on Sperm Quality

When comparing the sperm count, sperm motility, and sperm viability of male rats treated with Busulfan, Busulfan +Vit E, and Vit E alone for 28 days, as shown in table (2), there is a significant ($p < 0.05$) increase in sperm abnormality in the Busulfan group when compared to the control group, the busulfan +Vit E group, and the Vit E alone group. When comparing the busulfan + Vitamin E group to the busulfan group, there is a considerable improvement in sperm count, sperm motility, and sperm viability.

**Table 1.** Busulfan and vitamin E affect the body's weight and the testes.

| Groups | Body weight (g) | Testes weight (g) |
|---|---|---|
| **Control** | 325.45±5.273 a | 1.6487±0.1143 a |
| **Buselfan** | 277.164±8.573b | 1.1354±0.0835 c |
| **Buselfan+ Vit E** | 303.51±10.46 b | 1.5832±0.0684 b |
| **Vitamin E** | 330.193±12.46 a | 1.549±0.08845 a |

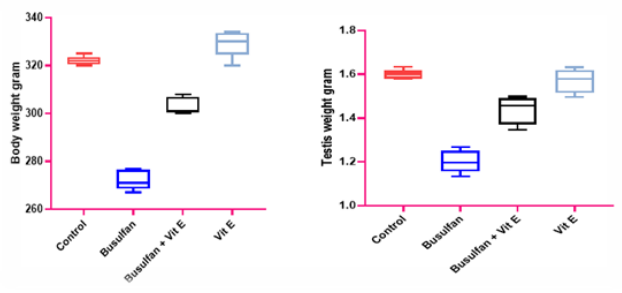


**Fig. 2.** Shows how busulfan and vitamin E affect the body's weight and the testes.

### 3.2. The influence of Buselfan and Vitamin E on the antioxidant activity of enzyme antioxidants

Table 3 illustrates that antioxidant enzyme levels were significantly lower in the Busulfan group compared to both the control group and the groups receiving Busulfan with Vitamin E or Vitamin E alone ($P < 0.05$). In contrast, the groups treated with Vitamin E, whether alone or in combination with Busulfan, exhibited a notable increase in these enzyme levels. The table details the variations in several enzymatic antioxidants related to the serum's antioxidant status. Rats treated with Busulfan showed significantly reduced levels of SOD, CAT, and GSH compared to control rats. However, when Vitamin E was administered alongside Busulfan, serum levels of SOD and CAT activity, as well as GSH levels, were significantly higher than those observed in the Busulfan-only group.

## 4. Discussion

This may have occurred as a result of Busulfan's cytotoxic effects on these organs as a result of the transfer of the alkyl group(s), the possible explanations include busulfan's cytotoxic effects and subsequent alkyl group transfer to a different cell type. While several factors contribute to cell death, DNA alkylation events may play a significant role [18]. Busulfan's alkylating properties lead to a partial wipeout of stem cells, The free radicals it generates are what do the actual damage, It would indicate that busulfan has an inhibitory effect on spermatogenesis, most likely by oxidative damage. Busulfan may have acted in part by elevating levels of ck18, a surface marker seen on Sertoli cells. Infertility and sperm dysfunction are linked to an increase in this marker [19]. Busulfan causes a decrease in both body weight and the weight of the testes in this investigation. It has been demonstrated that there is a one-to-one correlation between the size of a primate's testicles and the total number of germ cells in their bodies [20]. Busulfan was found to generate chromosomal abnormalities as well as dominant lethal mutations in sperm, as demonstrated by Bucci et al. [21].

This study's findings indicate a statistically significant (p 0.05) reduction in both the body weight and the weight of the testicles when compared with the group that served as the control. This finding agrees with previous research [22]. The fact that one of the side effects of busulfan administration is diarrhea and weight loss due to the injection. It disagrees with a study, that found [23] no significant difference in body weight between busulfan and control groups.

Infertility is one of the most significant challenges a married couple can face in their time together. The use of chemotherapy medications to treat cancer is one of the many factors that might impact the generation of sperm and increase the risk of infertility. Other factors include age, race, and gender. One of these medications that has a severe negative impact on the testicles is busulfan [24]. According to a study [25], these chemicals can potentially lower sperm concentration while producing free radicals and oxidizing germ cells in the testicles. This study examined how vitamin E protects rat sperm from damage caused by busulfan. We picked a four-week time frame to see if spermatogenesis could return in animals given busulfan.

**Table 2.** The effects of Buselfan and Vitamin E on several sperm parameters.

| Group | Sperm count ($10^6$/ml) | Sperm motility | Sperm viability | Sperm abnormality |
|---|---|---|---|---|
| **Control** | 73.48 ±4.732 | 70.45±6.265 | 74.21±4.287 | 5.90±2.312C |
| **Buselfan** | 10.72±3.854 | 13.54±3.578 | 16.29±2.985 | 40.35±3.632 |
| **Buselfan+Vitamin E** | 27.54±4.765 | 27.76±6.187 | 28.44±3.396 | 17.132±4.016 |
| **Vitamin E** | 78.43±6.248 | 75.54±8.138 | 76.19±5.913 | 4.091±2.567C |

**Table 3.** The effects of buselfan and vitamin E on enzymatic antioxidants.

| Group | CAT | GSH | SOD |
|---|---|---|---|
| **Control** | 68.945±9.528 | 32.198±6.345 | 96.831±3.319 |
| **Buselfan** | 30.537±3.741 | 13.419±1.213 | 89.70±3.036 |
| **Buselfan+Vitamin E** | 46.26±7.51 | 45.646±10.187 | 95.385±4.903 |
| **Vitamin E** | 71. 33±7.47 | 75.333±9.641 | 98.49±4.716 |

According to a study by a study [26], it took about four weeks for the effect of busulfan to distinguish between cells released from the seminiferous tubular lumen after busulfan treatment.

The antioxidant enzyme busulfan group had a considerable drop, consistent with previous study [27]. Highly cytotoxic and genotoxic, busulfan can induce various adverse effects [28]. Effects, both short- and long-term, including DNA damage, which triggers apoptosis or aging in a Cell type-dependent way. The reproductive system is just one bodily organ that might be negatively impacted by stress, Cancer treatment with busulfan has been shown to kill sperm by increasing their exposure to reactive oxygen species (ROS) [27].

## 5. Conclusion

The study emphasizes the harmful effects of Busulfan on body weight, testicular weight, and sperm quality in male rats, with significant reductions observed in these parameters compared to the control groups. Importantly, the addition of Vitamin E alleviated some of the negative impacts associated with Busulfan treatment, resulting in improvements in sperm count, motility, and antioxidant enzyme levels. These findings suggest that Vitamin E may act as a protective agent against the detrimental effects of Busulfan. Overall, the results highlight the importance of considering antioxidant supplementation in therapeutic settings involving Busulfan. Further research could investigate the mechanisms behind these protective effects and their potential clinical applications.

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