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L-carnitine treatment of adult rats with busulfan-induced oligospermia

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Abstract. The purpose of this research was to examine the effects of L-carnitine treatment on adult rats that had busulfan-induced oligospermia. In our experiment, we used twenty-one adult male rats, each weighing around 200 g. Three groups of rats were used for the experiments: The first set of subjects, called the control group, had DMSO administered intraperitoneally and 1 mL of pure water gavaged orally once daily. The second group received 20 mg/kg of busulfan (Bus) administered IP once daily along with a daily oral gavage of 1 ml of distilled water. The third set of subjects received a single I.P. of busulfan along with L-carnitine (L-car) and busulfan administered orally. One day after busulfan injection, L-carnitine were administered for a duration of 28 days at 350 mg/kg.bw. After dissecting, the epididymis and testis were meticulously removed and weighed, along with any excess fat and blood. The spermatozoa were taken from the removed caudal epididymis and incubated in PBS medium containing at 37C for 5 minutes, this used for counting and checking abnormalities. MDA and GSH were also estimated in testes tissues by using HPLC. The administration of BU resulted in a substantially testes weight, and relative testes weight ($p < .05$) as compared to the control group. testes weight, and relative testes weight were all markedly improved with L-carnitine administration as compared to G2. after administering BU, sperm count and motility were significantly lower ($p < .05$) compared to G1. There was a significant improvement in motility, sperm count, and abnormalities with L-carnitine administration compared to G2. after BU was administered, MDA were considerably higher and GSH significantly lower ($p < .05$) as compared to G1. The results of measuring oxidative stress indicators were dramatically improved with L-carnitine administration when contrasted with G2. In conclusion, Busulfan may have a negative impact on male fertility if taken alone, but when combined with L-carnitine, the drug's cytotoxic effects can be mitigated, protecting both testis and semen.

Highlights:

1. **L-carnitine Treatment: Significantly improved testis weight and sperm quality in busulfan-treated rats.**
2. **Oxidative Stress Reduction: L-carnitine mitigated increased MDA levels and restored GSH in testicular tissue.**
3. **Fertility Protection: Co-administration of L-carnitine reduced the cytotoxic effects of busulfan on male fertility.**

Keywords: L-carnitine, busulfan, oligospermia, male fertility, oxidative stress

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Introduction

The alkyl sulfonate class includes the bifunctional antineoplastic drug busulfan [1]. Because of its alkylating properties, it causes cancer cells to die by attaching itself to a specific DNA strand [2]. Busulfan is a very inexpensive chemotherapeutic medication used to treat leukemia. Following therapy, busulfan induced side effects in a number of organs, including those involved in reproduction. According to [3], young male patients treated with busulfan may have oligospermia, azoospermia, testicular atrophy, and ultimately infertility.

The reproductive system, in particular the epididymis, as well as high-energy-demanding tissues including muscles, skeleton, and heart have a high concentration of L-carnitine. Isolation of L-carnitine from cow muscle occurred in 1905. Of the L-carnitine that the body stores, around 75% comes from food and only 25% is made from scratch using lysine and methionine.

The processes of spermatogenesis, sperm maturation, and sperm motility are all significantly impacted by L-carnitine. According to [4], this tiny water-soluble particle plays a significant role in fat metabolism. The oxidation of long-chain fatty acids in mitochondria, which results in energy production, is a key process in which it participates. The release of carnitine esters from poorly metabolized acyl groups, storage of energy as acetylcarnitine, and modulation of the acyl-CoA/CoA ratio are further factors [5].

Compared to circulating levels (10-50 μm); [6], the concentration of L-carnitine in epididymal fluid and spermatozoa is about 2,000 times higher, ranging from 2 to 100 mm. The epididymal plasma receives free L-carnitine by way of the blood plasma. It accumulates as both free and acetylated carnitine in the spermatozoa after passively diffusing into them. According to [6], the content of L-carnitine in the epididymal lumen is directly correlated with the commencement of sperm motility. The diagnosis of obstructive azoospermia is another possible application of seminal free L-carnitine. Cases of post-epididymal blockage, like vas deference agenesis, are associated with very low carnitine contents [7].

Several pathophysiological disorders have been shown to be ameliorated by L-carnitine's antioxidant, anti-inflammatory, and anti-apoptotic properties [8]. The diagnosis of male infertility, however, is associated with reactive nitrogen and oxygen species (RNS and ROS). There are a number of strategies that have been developed to decrease or eradicate ROS and RNS generation in spermatozoa, which is very important for in vitro fertilization. Furthermore, spermatozoa naturally produce a number of

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ROS/RNS, including as nitric oxide (NO), hydrogen peroxide, and superoxide anion. In particular, NO plays a crucial role in controlling sperm energy and the acrosome response, both of which are essential for spermatozoa to achieve fertilization. It has been recently shown that mitochondria have a significant impact in the quality and likelihood of human sperm fertilization. Not only that, but NO and NO precursors boost mitochondrial energy generation, which in turn increases sperm motility [9].

This study aimed to investigate the L-carnitine therapy of adult rats with oligospermia induced by busulfan.

Method

We used twenty-one mature male rats (weighing about 200 g) in our experiment. To make a busulfan solution, the following steps were taken: dissolving busulfan (Sigma company) in DMSO (dimethyl sulfoxide); adding an equivalent amount of sterile water; and last, adding 20 mg/ml of busulfan [10]. The L-carnitine was prepared by dissolving it in distilled water to a concentration of 350 mg/kg.bw (Sigma) [11].

Three groups of rats were used for the experiments:

The first set of subjects, called the control group, had DMSO administered intraperitoneally and 1 mL of pure water gavaged orally once daily. The second group received 20 mg/kg of busulfan (Bus) administered intraperitoneally once daily along with a daily oral gavage of 1 ml of distilled water. The third set of subjects received a single I.P. of busulfan along with L-carnitine and busulfan administered orally. One day after busulfan injection, L-carnitine were administered for a duration of 28 days [4].

We weighed the rats, anesthetized with diethyl ether, and then killed them after the treatment period ended. After dissecting, the epididymis and testis were meticulously removed, along with any excess fat and blood. After removing the epididymis, it was chopped and placed in 1 ml of phosphate buffered saline (PBS, pH 7.2) according to [12]. For future testing, the homogenized testes tissues were frozen at -20°C with the liquid remaining in the tubes.

To determine the sperm count, the rat's whole epididymis was chopped and incubated for 5 minutes at 37°C in PBS medium. Using a Neubauer hemocytometer, the concentration of sperm was manually evaluated [12].

In order to evaluate the motility of the sperm, the spermatozoa were taken from the removed caudal epididymis and incubated in PBS medium containing at 37C for 5 minutes. Motility was defined as the presence or absence of movement in spermatozoa.

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Using computer-assisted sperm analysis, the quantities of total spermatozoa, motile spermatozoa, velocities of spermatozoa, types of motility, and percentages of motile spermatozoa were determined for each treatment group [13]. To identify sperm abnormalities, a single drop of sperm suspension is applied to the tip of a glass slide, spread out, and then stained with Eosin, nigrosin, or both.

For oxidative stress estimation, to get rid of nuclei and debris, the homogenate was spun in a chilled centrifuge at 2,000 × g for 15 minutes at 4°C. The following tests were conducted using the collected supernatant: oxidative stress (MDA and GSH) using HPLC equipment.

The data was analyzed statistically using the GLM, which was developed by the Statistical Analysis Systems Institute (SAS).

Results

The administration of BU resulted in a substantially testes weight, and relative testes weight ($p < .05$) as compared to the control group, as indicated in Table 1. testes weight, and relative testes weight were all markedly improved with L-carnitine administration as compared to G2.

Table 1. parameters of testes in studied groups

Parameter	G1	G2	G3
Testes wt (gm)	2.49 ± 0.14 A	1.07±0.41 C	1.91±0.52 B
Testes to body weight ratio (%)	1.02 ± 0.13 A	0.34± 0.17 B	1.0±0.23 A

Table 2 shows that after administering BU, sperm count and motility were significantly lower ($p < .05$) compared to G1. There was a significant improvement in motility, sperm count, and abnormalities with L-carnitine administration compared to G2.

Table 2. Sperm parameters in studied groups

Parameter	G1	G2	G3
Sperm count (10^6)	120	17	116
Motility (%)	82	18	76
Abnormalities (%)	3	19	4

Table 3 shows that after BU was administered, MDA were considerably higher and GSH significantly lower ($p < .05$) as compared to G1. The results of measuring oxidative stress indicators were dramatically improved with L-carnitine administration when contrasted with G2.

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Table 3. Oxidative stress in studied groups

Parameter	G1	G2	G3
MDA (nmol/g tissue)	17.28 ± 1.36 C	29.7±3.23 A	21.42±2.42 B
GSH (µmol/g tissue)	13.84±0.94 A	6.92±1.57 C	10.98±1.73 B

Discussions

Although many people do manage to beat cancer, it remains one of the leading killers. Despite their usefulness in cancer therapy, anticancer medications come with their fair share of adverse effects. These anticancer medications may have serious side effects on male fertility, including infertility. Accordingly, several research have focused on creating pharmaceutical or herbal remedies to alleviate or lessen its negative effects [3]. Although it is used to treat cancer, the cytotoxic effects of the anticancer medication busulfan have caused a number of problems with the male reproductive system.

A decrease in spermatozoa number, motility, and speed was seen in this research after injection of busulfan. Busulfan exhibited a decrease in testes weights along with an increase in DNA metabolites and oxidative stress markers. Consistent with earlier research [14][15], these findings were verified. Positive expression of caspase-3 in testis tissue was another indicator of an apoptotic impact in the group treated with busulfan. With this finding, [16] was reached as well. The discharge of free radicals might be the cause of Busulfan infertility. According to a study by [4], sperm genomes were impacted by free radicals, leading to alterations that are permanently harmful. Busulfan may also induce sperm abnormalities by increasing the Sertoli cell marker (ck18), according to another theory [17].

Previous research using cells in vitro found that busulfan reduced cell proliferation and blocked cell life, which in turn triggered apoptosis and caspase 3 cleavage [18].

However, in this research, oxidative stress and semen parameters were both improved by administering L-carnitine. Additionally, the groups treated with L-carnitine had their caspase-3 expression levels measured in the testes. The complex function that L-carnitine plays in regulating cellular energy and overall homoeostasis is due to its status as a nitric oxide precursor. According to [19], the greater levels of NO seen in the testes homogenate of rats treated with busulfan are likely due to a decrease in sperm counts and metabolism.

There is strong evidence that therapy with L-carnitine may prevent busulfan infertility. Our results show that L-carnitine therapy enhances oxidative stress, cell energy, and sperm quality. Also, caspase-3 expression was negative in the L-carnitine-

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treated group. The antioxidant activity of L-carnitine may explain its protective effect; by eliminating the harmful acetyl-coA and replacing it with a different fatty acid in the cell membrane, it shielded the spermatozoa's plasma membrane from oxidative stress caused by harmful substances [20]. Fatty acids are the primary energy source for spermatozoa, however they may employ a variety of substrates [21]. According to [5], acetylcarnitine is synthesized in abundance in the epididymal fluid by the oxidation of fatty acids in the presence of a significant reservoir of L-carnitine, suggesting that it may serve as an energy source for spermatozoa. In addition to regulating the Krebs cycle, L-carnitine supplies it with free coA [22].

Lenzi et al. [23] proposed that L-carnitine improved sperm count and quality by increasing the quantity of spermatids and decreasing phagocytosis of gametes, which is in accordance with our findings. In addition, l-carnitine's antioxidant action improves sperm survival and chromatin integrity by increasing sperm glucose uptake [24].

Conclusion

Busulfan may have a negative impact on male fertility if taken alone, but when combined with L-carnitine, the drug's cytotoxic effects can be mitigated, protecting both testis and semen.

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