AMELIORATION EFFECTS OF VITAMIN E ON REPRODUCTIVE SYSTEM IN MALE RATS EXPOSED TO BUSULFAN

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ABSTRACT

Twenty four male rats ten weeks old, They weigh between 250-300 grams, were divided into four groups. First group as control, received an intraperitoneal injection of Dimethyl sulfoxide (DMSO) daily for four weeks (also use as a solvent for other experiment materials). Second group receive only single dosage of Busulfan 40mg/kg BW/IP. Third set was received single dose of Busulfan as 40 mg/kg BW/IP & Vitamin E as 100 mg/kg BW/IP daily for 4 weeks & the fourth group receive only Vitamin E as 100 mg/kg BW/IP daily for 4 weeks. After the end of 4 weeks the Animals were sacrificed & the blood was collected for hormonal measuring & epidydimis was collected for sperm count & histological examination.

The outcomes demonstrate that busulfan prompts significant rise in serum FSH & LH level although Testosterone level was significantly reduced. Additionally, busulfan initiated significant reduction in sperm count, motility & viability & significant rise in sperm abnormality. Alternatively, Co-administration of vitamin E with busulfan lead to ameliorate levels of hormone as well as sperm activity.

Keyword: Busulfan, vitamin E, reproductive hormones.

I. INTRODUCTION

Infertility is described as a couple's failure to conceive after at least a year of sexual activity, with men accounting for nearly half of infertility cases [1]. Infertility is caused by a variety of causes including lifestyle, medications, toxicants, & infections [2]. Busulfan is compound that cause reproductive toxicity. Chronic myelogenous leukemia & blood diseases are treated with Busulfan. [3].

Busulfan showes to reduce sperm motility, viability, & number. This medication reduces the size & weight of the testicles. Busulfan is a chemotherapeutic & cytostatic agent extensively used to eliminate autologous germ cells from testes of animal models. [4]. Chemotherapy & radiotherapy in Busulfan are associated with many changes in the male reproductive system, which is a white crystalline powder, its is an alkylation agent & a cytotoxic drug back to the alkyl sulfonate group [5]. Busulfan, has been given in greater dosages earlier to a bone marrow or stem cell transplant for other kinds of cancer [6,7]. Busulfan - induced oxidative stress outcomes from an disparity between the production of reactive oxygen species (ROS) & the shielding effect of the antioxidant system in charge for their neutralization & elimination. An extra of reactive oxygen species principals for the pathological reaction that leads to destruction of the cells & tissues. Sperm are particularly vulnerable to the harmful effects of ROS. Oxidative stress affects their activity, terminates DNA structure, hurries apoptosis, all of which subsequently decreases their numbers, spoils movement & normal shape development, & impairs function. This leads to conflicts in fertility. Reactive oxygen species are eradicated by some antioxidants such as vitamin E (9). Vitamin E is a group of 8 fat - soluble compounds having 4 tocopherols & 4 tocotrienols. (10). It is a fat – soluble antioxidant that guards cell membranes from reactive oxygen species.(11)

The connotation between cancer risk & vitamin E has been studied in several epidemiological revisions. The anticancer special effects of vitamin E has been qualified mostly to its anti - oxidant, anti - inflammatory, anti - proliferative, anti - angiogenic, immune modulatory mechanisms [10]. Vitamin E (a – tocopherol) placed
commonly in cell membranes. This dominant antioxidant extinguishes superoxide anions & free hydroxyl radicals thereby reducing lipid peroxidation initiated by ROS in plasma membranes [12]. Therefore, it guards the cell membrane from injury triggered by ROS.

The current study designed to estimate the consequence of Vitamin E on testicular tissue in busulfan-treated rats. Assessments were based on criteria for sperm quality, reproductive hormones & tissue samples.

II. MATERIALS & PROCEDURES

In this paper, 24 in good physical shape male rats was saved in typical circumstances of temperature 22 ± 2 °C, humidity 30 – 60 % & a period of light of 14 hours & 10 hours of dark. The rats was arbitrarily separated into 4 groups of 6 rats per group: the control group gived D M S O (0.1 ml daily, i.p.). The 2nd group gived a single dosage of Busulfan 40 mg / kg, i.p. The 3rd group received a single dose of Busulfan 40 mg/kg, i.p. & 0.1 ml of vitamin E (100 mg / kg, i.p.). The 4th group gived 0.1 m L of vitamin E (100 mg / kg, i.p.). All events continued for 28 days.

Left testes were secure in 10 % salt-water for 72 h, laterly specimen were dried up, cleaned, & paraffin place in. Paraffin sections (6 - 7 μm thick) & stained with (H & E) dye, were for histological examines using an optical microscope & imaged with a digital camera. All data were statistically analyzed by using software of IBM (SPSS, version 22). One - way A N O V A test were done to compare between groups of study & least significant differences (L S D) used to identify significance of the differences between means & P value less than 0.05 were considered significant. Obtained results were expressed as the mean plus minus standard error.

III. RESULTS

The outcomes in table (1) presented significant (p < 0.05) decrease in the sperm count, sperm motility, sperm viability & significan rise in sperm abnormality in Busulfan group when relate with control group, busulfan + Vitamine E group & Vitamine E alone group. Also, there is significant rise in busulfan + Vitamine E group when relate with busulfan alone group.

Table 1: Effect of Vit E on sperm count, motility, viability & abnormality in Buaelfan treated male rat. (Mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Sperm count (10^9/ml)</th>
<th>Sperm motility (%)</th>
<th>Sperm viability (%)</th>
<th>Sperm abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>70.67±5.854B</td>
<td>69.17±8.280A</td>
<td>72.83±3.764B</td>
<td>6.00±1.414C</td>
</tr>
<tr>
<td>Busulfan</td>
<td></td>
<td>11.83±2.639D</td>
<td>15.83±3.920C</td>
<td>17.00±3.162D</td>
<td>43.67±4.719A</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td>77.83±5.492A</td>
<td>74.33±7.033A</td>
<td>77.17±4.708A</td>
<td>3.83±1.472C</td>
</tr>
</tbody>
</table>

The results presented in Table 2 presented a significant (p < 0.05) reduction in testosterone level & a significant (p < 0.05) rise in LH & FSH in the Busulfan group compared to the control group, busulfan + vitamin E, & vitamin E group alone.

Table 2: Effect of Vit E on serum hormone level in Busulfan treated male rat. (Mean ± SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Testosterone (ng/mL)</th>
<th>FSH (ng/mL)</th>
<th>LH (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.721 ± .254 B</td>
<td>1.856 ± .224 C</td>
<td>1.728 ± .072 BC</td>
</tr>
<tr>
<td>Busulfan</td>
<td></td>
<td>.810 ± .118 D</td>
<td>3.545 ± .885 A</td>
<td>2.768 ± .717 A</td>
</tr>
<tr>
<td>Busulfan+Vitamin E</td>
<td></td>
<td>1.263 ± .355 C</td>
<td>2.993 ± .985 AB</td>
<td>2.035 ± .389 B</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td>2.480 ± .531 A</td>
<td>2.343 ± .735 BC</td>
<td>1.862 ± .247 BC</td>
</tr>
</tbody>
</table>
Histological Picture & Changes of the Testes

The histological section was read under a magnification of x10 & x40. Examination of the tissues of the normal testes of rats shows that the seminiferous tubules are spherical or oval in shape, lined with germinal epithelium consisting mainly of spermogenic cells that are in the gradual stages of spermatogenesis in the lumen of the seminiferous tubules, between the seminiferous tubules there are interstitial spaces with the group that produce interstitial Leydig cells.

Histological examination in this study displayed a reduction in the width of the seminiferous tubules & the depth of the seminal epithelium. The seminiferous tubules also suffer from expansion with some nonappearance in the process of spermatogenesis in groups of male rats treated in the second group.

Normal seminiferous tubules appear with increased spermatogenesis & spermatogenesis in groups of male rats treated with vitamin E in the fourth group.

Figure (1) Photomicrograph of rat testis shows normal histological features in control group, normal histological structures of seminiferous tubules (black arrow) & interstitial tissues (white arrow) & typical seeming spermatogenesis activity in seminiferous tubules (blue arrow). (Hematoxylin & Eosin, X 10)

Figure (2) Image of seminiferous tubule of control group, reveals normal oval to rounded seminiferous tubule are seen.
Normal histological structure of the germinal epithelium. Spermatozoa (black arrow) are filling the lumina of the tubule. There are different types of spermatogenic cells lining the tubule including spermatogonia (white arrow), primary spermatocytes (yellow arrow), spermatids (blue arrow), & spermatozoa. (Hematoxylin & Eosin, X40)

Figure (3) Image of busulfan treated group display damage in testicular tissues. The seminiferous tubular atrophy & germinal epitheliums aplasia characterize no spermatogenic activity. The most of spermatogonia (black arrow) & primary spermatocytes cells (white arrow) are destroyed in busulfan treated group, the interstitial tissue were atrophied (blue arrow) with few Leydig cells. (H&E stain X10)

Figure (4) Image of busulfan treated group showing the seminiferous tubular atrophy, reduced width of germinal epithelium & vacuolar space on the basement membrane of the seminiferous tubules were detected (black arrow). The spermatogonia & primary spermatocytes cells are destroyed, no spermatozoa in the lumen of the seminiferous tubule. (H & E, X40)
Figure (5) Image of seminiferous tubules of group treated with busulfan & Vitamin E, displayed almost normal arrangement of germinal epithelium (white arrows) & represent reestablishment of spermatogenesis (black arrow), mild congestion of interstitial blood vessel (blue arrow). (H & E, X10)

Figure (6) Image of seminiferous tubules of group treated with busulfan & Vitamin E, displayed slight reduction of germinal lining represented by the view of moderate number of germinal cells (blue arrows) with a number of spermatids in the lumen of the recovered seminiferous tubules (black arrow), congested interstitium with average Leydig cells (white arrow) & presence of vacuolation of the seminiferous tubules (white arrow). (H & E, X40)
IV. DISCUSSION

There are many aspects that can disturb sperm production and lead to infertility, one of the significant aspects is chemotherapy for cancer. Busulfan is one of these medications that affects the testicles. (13). These agents can decrease sperm concentration while producing free radicals and oxidation of germ cells in the testes. (14).
In table (1) outcomes presented a significant reduction in busulfan group compared to the other group (p < 0.05), in sperm count, motility, viability & significant rise in sperm abnormality. Our findings were agreement with other research(15,16). Our results showed that administration of Busulfan in a single dose & daily give of Vit E for 4 weeks can pointedly decrease busulfan – mediated demolition of the testis. This is since the Vit. E reduce testicular tissue injury produced by cytotoxic mediators by growing the appearance of genes connected to antioxidants. In the male reproductive system, the antioxidant property of Vit E acts to prevent the unhelpful effects of free radicals in the testes & sperm (17,18). In addition, some revisions have shown that vitamin E is in effect in self-protective the testiciles from injury produced by oxidative stress & moderating this injury can be accomplished by treatment with vitamin E (19).

In our research as shown in (Table 2) we found that LH & FSH levels were significantly increased (P≤0.05) while testosterone levels were decreased in busulfan-treated rats as compared to the other groups. Busulfan is a drug that greatly affects the testiciles. It causes a pronounced increase in apoptosis & affects spermatogenesis. Busulfan has toxic effects on the spermatic epithelium of rats & causes infertility(13). Mainly by killing sperm stem cells. A primary gonadal defect is caused by a testicle. Damage has a detrimental effect on spermatogenesis &/or defect in Leydig cells. (20). As mentioned above, the effect of busulfan & the elevated FSH level in this study may be due to impaired spermatogenesis, while the low testosterone levels associated with elevated LH may occur as a result of Leydig cell dysfunction. (21).

Exposure to chemotherapy & cytotoxic radiotherapy leads to associated elevation of FSH levels with decreased sperm count, & thus, suppressive effects of testosterone analogs & gonadotropins on sperm. Moreover, chemotherapy is often used with increased testicular weakness & damage to the epithelial microbiota. To make treatment of male infertility possible after cancer treatment (22). Also, the accumulation of free radicals & thus oxidative injury in the Leydig cells of the testis by specific chemical oxidants (eg busulfan) may degrade their response & performance of testosterone synthesis. (23).

The results of the current paper displayed that busulfan decreases germinal epithelium raise in rats. There was elimination & abnormality in the sperm cells with exfoliation in the lumen of the seminiferous tubules. Thus, the reduction in the width of the gothic tubules of the epithelium might be a significance of the degenerative effects of busulfan(24). Others displayed that busulfan harvests free radicals that straightly affect DNA. The DNA destruction leads to chromosomal abnormalities & regularly lethal mutations in sperm. Promotion of this indicator primes to humble spermatogenesis, infertility & reduced sperm motility by reducing flagellum length(15).

REFERENCES


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