Effect of Subchronic Vitamin E Treatment on the Testes of Male Albino Rats

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Abstract: Vitamin E is widely used as a drug supplement in many diseases. The aim of these investigations was conducted to study vitamin E supplement effect on testicular tissues in male albino rats after 90 days of repeated treatment and after stopping treatment for 45 days. Seven groups of six male rats were orally administered 500, 1000 and 2000 mg/kg body weight vitamin E daily and the control received distilled water for 90 days. Testosterone and histological examination of testes were assessed. The elevation of the number of seminiferous containing sperm and not containing sperm was counted. The rats treated with two higher Vitamin E supplement dose treatment caused a significant increase in testosterone and seminiferous containing sperm. Therefore, oral administration of vitamin E supplement in rats for 90 days was safe for the testis and has a spermatogenic effect. The androgenic effect of vitamin E persists even stopping treatment.

Keywords: Vitamin E; testes histology; testosterone; fertility.

1. INTRODUCTION

Infertility is a major clinical problem, affecting people medically and psychosocially (1). Reduced male fertility can be the result of congenital and acquired urogenital abnormalities, infections of the male accessory glands, increased scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors (2). In 40–60% of cases the only abnormality is the semen analysis and there is no relevant history or abnormality on physical examination and endocrine laboratory testing (idiopathic male infertility) (3). Semen analysis reveals a decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). Usually, these abnormalities come together and are described as the OAT-syndrome (oligo-astheno-teratozoospermia) (4). Numerous studies have reported beneficial effects of antioxidant drugs and supplement on semen quality and treatment of male infertility (5,6) but there is no well-defined therapeutical protocol.

Vitamin E has been effectively used as supplement with drugs for treatment of many diseases (7). Use of vitamin E has increased considerably in last decades in view of the increased awareness as regards its antioxidant and androgenic potential. Many studies have been studied in the androgenic activity of vitamin E in the induced testicular toxicity (8,9). It was also known to induce a protective effect for testicular toxicity (11,12). Takhshid, Tavasuli, Heidary, Keshavarz and Kargar (13) reported an increase in serum testosterone level in rats treated with vitamin E and reducing oxidative stress in testes. (Aybek, Aybek, Rota, Şen and Akbulut (14), reported the protective effect of vitamin E on testicular function and in increasing male capacity. Since some male patient is prolonged by using vitamin E, the effect of prolonged use in reproductive capacity has to be investigated.

2. MATERIALS AND METHODS

2.1. Experimental Animals

Forty two male albino rats weighing 100-120 g were kept for about 15 days before the onset of the experiment, under observation to exclude any intercurrent infection and to acclimatize the laboratory conditions. The animals were kept in polyethylene home cages with good aerated covers at normal
atmospheric temperature (25±5°C) and at normal daily 12 hrs dark/light cycles. They were fed commercial food pellets and provided with tap water ad libitum. The experiment was performed in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines (17).

2.2. Methods
The animals divided into seven groups, each of 6 rats.

Control group: received by intra-gastric gavage tube distilled water for 90 days

Vitamin E 500 group: treated orally with 500 mg/kg b.wt. vitamin E for 90 days by intra-gastric gavage tube.

Vitamin E 1000 group: treated orally with 1000 mg/kg b.wt. vitamin E for 90 days by intra-gastric gavage tube.

Vitamin E 2000 group: treated orally with 2000 mg/kg b.wt. vitamin E for 90 days by intra-gastric gavage tube.

SVIt E 500 group: treated orally with 500 mg/kg b.wt. vitamin E by intra-gastric gavage tube for 90 days, then left for 45 days.

SVIt E1000 group: treated orally with 1000 mg/kg b.wt. vitamin E by intra-gastric gavage tube for 90 days, then left for 45 days.

SVIt E2000 group: treated orally with 2000 mg/kg b.wt. vitamin E by intra-gastric gavage tube for 90 days, then left to recover for 45 days.

The blood samples were collected from the medial retro orbital venous plexus of mice with capillary tubes (Micro Hematocrit Capillaries) under ketamine anesthesia for clinical chemistry assays into a plain gel tube and were standing for an hour at room temperature to obtain serum aliquots. Then the tubes were centrifuged at 5000 r.p.m for 10 min and the top yellow serum layer was populated off for testosterone analysis. After the blood sampling the animals were scarified by dislocation and dissected to obtain the organs for histological assays.

2.3. Determination of Serum Testosterone Concentration
Serum testosterone (ng/ml) was determined in the serum by enzyme Immunoassay kit (Meddix Biotech Inc., 420 Lincoln Centre Drive, Foster City, CA 94404, USA, and Catalog Number: KEF4057) according to (18).

2.4. Histological Preparation
Immediately, after the animals were sacrificed testis of each animal were quickly excised, washed with normal saline for the removal of the blood, which might obstruct the process of fixation, blotted with filter paper, weighted and rinsed in ice-cold saline and fixed in 10% formaldehyde overnight and they were processed using standard histological methods as described by El-Hak (19). The spermatogenic seminiferous tubules and non spermatogenic seminiferous tubules counted.

2.5. Statistical Analysis of Data
The data were analyzed using SPSS 11.0 for Windows. The statistical significance of differences was evaluated by using one-way analysis of variance (ANOVA) followed Post-hoc Dunnett was used to make the comparison between means. All the data were expressed in mean ± standard error (SE) and the p-value less than 0.05 was considered significant. The percent was compared between groups using nonparametric analysis of variance (Kruskal-Wallis test) followed by Mann-Whitney U test comparing groups against each other.

3. Results
The effect of vitamin E supplementation on the testosterone hormone in control and different treated groups was demonstrated in table (1). There was a significant increase \((p<0.005)\) in testosterone level when the rat received vitamin at dose 1000 and 2000 mg/kg b.w.t. for 90 days, respectively. On the other hand, in the stopping treatment groups, testosterone showed an nonsignificant change compared to the control group.
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There was an increase in sperm containing seminiferous tubules when the rat received vitamin at dose 1000 and 2000 mg/kg b.wt. for 90 days, respectively, compared to the control percentage. The percentage of sperm containing seminiferous tubules in the stopping treatment group and treated rats with 500 mg/kg b.wt. for 90 days vitamin E showed no difference to the control group percentage table (1).

The seminiferous tubules of control and treated rats showed normal and a small number of abnormal seminiferous (Fig 1). The normal seminiferous showed normal arrangement of germinal epithelium spermatogonia and Sertoli cells resting on an intact basement membrane with typical structure of primary spermatocytes, round, elongated spermatids and late stage sperms. The abnormal seminiferous appeared with sloughing of germinal epithelium in the lumen and vacuolation in the germinal epithelium (Fig. 1-B & 1-F).

Fig(1): (A): Testis section of a control rat showing normal appearance of the seminiferous tubules (ST) with normal spermatogenesis showing spermatagonida (SG), primary spermatocytes (PS), spermatids (SD), (HX & E., 400 X). (B): Testis section of treated rat with 500mg/kg vitamin E for 90 days showing seminiferous tubules with vacuolation (V) and sloughing of the germinal epithelium in the seminiferous tubes lumen (S). (HX & E., 400 X). (C): Testis section of treated rat with 1000mg/kg vitamin E for 90 days showing seminiferous tubules (ST) with normal spermatogenesis (HX & E., 200 X). (D) Testis section of treated rat with 2000mg/kg vitamin E for 90 days showing seminiferous tubes (ST) with normal spermatogenesis (HX & E., 400 X). (E): Testis section of treated rat with 500mg/kg vitamin E for 90 days and left for 45 days after treatment showing seminiferous tubules (ST) with normal spermatogenesis (HX & E., 400 X). (F) Testis section of treated rat with 2000 mg/kg vitamin E for 90 days and left for 45 days after treatment showing seminiferous tubules with vacuolation (V) and sloughing of the germinal epithelium in the seminiferous tubes lumen (S). (HX & E., 400 X).

Table 1. Effect of treatment with vitamin E (500, 1000 and 2000 mg/kg) on testosterone hormone level (ng/ml) and percentage of sperm and non sperm containing tubules of treated rats 90 days and stopping treatment groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testosterone hormone (ng/ml) Mean ±S.E</th>
<th>Percentage of seminiferous tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sperm contain</td>
</tr>
<tr>
<td>Control group</td>
<td>1.18 ± 0.13</td>
<td>94.72%</td>
</tr>
<tr>
<td>Vitamin E 500 group</td>
<td>1.06± 0.145</td>
<td>84.86%</td>
</tr>
<tr>
<td>Vitamin E 1000 group</td>
<td>1.82 ± 0.129*</td>
<td>98.71%*</td>
</tr>
<tr>
<td>Vitamin E 2000 group</td>
<td>2.05±0.133*</td>
<td>98.11%*</td>
</tr>
<tr>
<td>SVit E 500 group</td>
<td>1.58± 0.110</td>
<td>94.82%</td>
</tr>
<tr>
<td>SVit E 1000 group</td>
<td>1.17± 0.046</td>
<td>94.64%</td>
</tr>
<tr>
<td>SVit E 2000 group</td>
<td>1.06± 0.044</td>
<td>94.73%</td>
</tr>
</tbody>
</table>

(*) represent a significant difference between the control and treated groups.
4. DISCUSSION
In the present study, treatment of rat with different doses of vitamin E for 90 days and stopping treatment for 45 days were investigated for alteration the testicular tissues and function in terms of serum testosterone level. In our study, treatment of vitamin E for 90 days caused increased spermatogenesis in the seminiferous tubules. In accordance with our finding, vitamin E can positively alter the reproductive steroid levels. Testosterone level is considered an essential indicator of testicular endocrine function (20). The result of this investigation confirmed that vitamin E has beneficial effects on male reproductive tissue and function by increasing testosterone level and appearance of normal testes tissue. These fertility properties may be due to the androgenic and the antioxidant activities of vitamin E (21, 22) and may be due to the positive correlated effect of vitamin E on testosterone and the higher expression level of steroid hormone biosynthesis enzymes (23).

The results of the present study also show that vitamin E not affect the histological structure of testes. Vitamins E is essential for normal spermatogenesis (24) and that in the absence of such vitamins, the animals showed dysfunction of the testis germinal layers (25). Supplementation of vitamins E has been established to reduce testicular ROS and restore normal testicular function in cadmium-exposed rats (26). Histological examination of rat testes, which received the vitamins E with daily dose (500, 1000 and 2000) mg/kg b.wt. For ninety days and after stopping the treatment. These results are being agreement to (27, 28).

Our finding shows that treatment with vitamin for a long time and large dose has no adverse effects on male reproductive system. Oral administration of vitamin E supplement in rats for 90 days has a spermatogenic effect and increase the fertility that persists even stopping treatment.

5. CONCLUSION
The present data suggest that vitamin E oral supplement with higher dose is safe and benefit the testicular endocrine and exocrine function. Further investigation is needed to determine the exact mechanism of increasing male fertility.

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Effect of Subchronic Vitamin E Treatment on the Testes of Male Albino Rats


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