EFFECT OF VITAMIN E ON SEXUAL EFFICIENCY IN MALE RATS TREATED WITH CADMIUM

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ABSTRACT

To examine the possible protective role of vitamin 'E' 500 mg/kg diet as antioxidant against cadmium induced oxidative stress, 20 male albino rats (3-4 months old) were exposed during 2 months to oral intake of cadmium 25 mg/L (as cadmium chloride) in drinking water, cadmium for 2 months associated with vitamin E (500 mg/kg diet) given at the second month, cadmium along with vitamin E for 2 months, or has been left as a control group. The results showed that cadmium produced no changes in body weight, testicular or prostatic weights. Epididymis and seminal vesicle weights with sperm count and the percentage of live sperms were decreased significantly, with an increased in the percentage of dead and morphologically abnormal sperms. Vitamin E, on the other hand, increased the percentage of live sperm and decreased the percentage of dead and morphologically abnormal sperm caused by cadmium. It is concluded that, vitamin E supplementation decreased the cadmium effect particularly when it is administered along with cadmium from the first day of experiment.

تأثير فيتامين هـــ على الكفاءة التناسلية في ذكور الجرذان المعاملة بالكادميوم

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الخلاصـــــة

لغرض معرفة الدور الوقائي لفيتامين هـــ (500ملغم / كغم عليقة) كمانع أكسدة ضد الكرب التأكسدي الذي يسببه الكادميوم, أخذ 20 جرذا أبيضاً بعمر 3– 4 أشهر وأعطــي كلوريد الكادميوم (25 ملغم / لتر) مع ماء الشرب لمدة شهرين, كذلك تم إعطاء كلوريــد الكادميوم لمدة شهرين صاحبها إعطاء فيتامين هـــ (500ملغم / كغم عليقة) منـــذ بدايـــة الشهر الثاني، أو أعطي الكادميوم سوية مع فيتامين هـــ من البداية ولمدة شـــهرين، أمـــا المجموعة الأخرى فأعتبرت مجموعة سيطرة. أظهرت النتـــائج أن الكـــرب التأكـــسدي المستحث بالكادميوم لم يسبب أي تغير فـــي وزن الجـــسم ووزن كـــل مـــن الخـــصية والبروستات. أما وزن البربخ والحويصلة المنوية وكذلك عدد النطف والنـــسبة المئويـــة للنطف الحية فقد انخفضت معنوياً, مع ازدياد في النسب المئوية للنطف الميتة والمشوهة. من ناحية أخرى, فقد سبب فيتامين هـــ زيادة معنوية في النسبة المئوية للنطف الحية مع

خفض في النسب المئوية للنطف الميتة والمشوهة. وقد أستنتج من الدراسة بأن فيتــامين هـــ قد عمل على التقليل من تأثير الكادميوم وخاصة عند استخدامه سوية مع الكـــادميوم منذ البداية.

INTRODUCTION

Cadmium, released from automobile exhausts, fossil fuels, industrial and domestic processes, is ubiquitous environmental pollutants. Human exposure occurs through diet, drinking water, inhaling the polluted air and smoking (1).

Cadmium accumulates in the human body for a long time even after minimal exposure and has severe toxic effects. Cadmium has been linked to osteomalacia, hepatotoxicity, renal toxicity, neurotoxicity as well as infertility and cancer (2). It is known to induce oxidative stress (3) and lipid peroxidation (LPO) by stimulating the production of superoxide anions (4). Within the cells, cadmium suppresses antioxidants such as superoxide dismutase or glutathione peroxidase (5). Free radicals then accumulate, leading to cell damage, aging and the development of chronic diseases.

In recent years, there has been an increasing interest in the contribution of occupational and environmental exposures to toxic pollutants to declining sperm concentrations and human male infertility (6).

An increase in oxidative damage to sperm membranes, proteins, and DNA is associated with alterations in signal transduction mechanisms that affect fertility (7). In addition, LPO has been reported to be accelerated among defective spermatozoa exhibiting high levels of reactive oxygen species and that α-tocopherol could reverse the functional consequences of LPO (8). Alpha-tocopherol is a chain-breaking antioxidant that exists in cell membranes (9). It eliminates lipid peroxyl and alkoxyl radicals, suppresses the chain reaction of LPO and promotes the production of scavenger antioxidant enzymes (10).

The current study was undertaken to characterize the response effects of cadmium on body weight, testicular weight, epididymis weight, accessory glands weights, and sperm quality in male rats, and to examine if vitamin E can protect changes induced by cadmium.

MATERIALS AND METHODS

Experimental design: Twenty male albino rats were obtained from the animal house of the veterinary medical college-university of Mosul, at 3-4 months of age, weighing 180-220 g. They were housed in polypropylene cages under controlled conditions of temperature (24-26 'C) and lighting (12 hours light/12 hours dark). The rats were supplied a standard pellet diet and tap water *ad libitum.* The animals were randomly divided into four groups (5 rats/group). The first group received tap water served as control. The second group received 25mg/L cadmium chloride in drinking water daily for 2 months (11). The third group received 25 mg/L cadmium chloride in drinking water daily for 2 months associated by feeding of vitamin E 500 mg/kg diet from the next second month (12, 13). In the fourth group, rats were fed for 2 months 500 mg of vitamin E/kg diet along with 25 mg/L cadmium chloride in drinking water.

Sample Collection and Analysis: At the end of the experiments, animal's weights were recorded. Animals were sacrificed by ether administration. The abdomen was incised and both testes were dissected out and their weights were

recorded. After drying with the surface of filter paper, the weight of the testis was recorded. Epididymal, seminal vesicles and prostate weight were also recorded. Epididymis was dissected out, sectioned and immediately the content of the head was squeezed gently in clean watch glass contained 9.8 ml. buffer formalin with 0.1 ml. eosin (5%). This was used for counting the sperm using hemocytometric technique (14, 15). The percentage of live, dead and morphologically abnormal sperms were counted in smear prepared from epididymal tail content by using eosin-nigrosin stain diluted with 3% sodium citrate (16).

Statistical Analysis: The results were expressed as mean ± SD. Our data were analyzed statistically using one-way analysis of variance. Group differences were determined using Duncan multiple range test. Statistical significance was considered at $p < 0.05$ (17).

RESULTS

The effects of cadmium and vitamin E treatment on body weight and (testicular, Epididymal, Seminal Vesicle, and Prostate weight) in albino male rats are presented in table (1).

Table 1: Effect of 60 days oral administration of cadmium (Cd^{2+}) and vitamin E on body weight and (Testicular, Epididymis, Seminal Vesicle, and Prostate weight) in albino male rats.

Treatment	Body Weight (g)	Testicular Weight (mg)	Epididymal Weight (mg)	Seminal Vesicle Weight (mg)	Prostate Weight (mg)
Control	a 307.20 ± 52.57	a 456.30 ± 28.53	a 181.38 ± 9.99	a 188.25 ± 39.04	a 415.84 ± 60.57
Cd^{2+}-Treated (25 mg/L. in drinking water)	a 328.20 ± 34.54	a 419.91 ± 66.29	b 140.37 ± 18.64	b 136.82 ± 37.00	a 385.00 ± 44.31
Cd^{2+} for 2 months + Vitamin E for 1 month (500 mg vitamin E/kg. diet)	a 324.80 ± 34.39	a 428.92 ± 42.72	b 150.13 ± 17.00	ab 159.47 ± 19.69	a 421.42 ± 39.65
Cd^{2+} for 2 months + Vitamin E for 2 months (500 mg vitamin E/kg. diet)	a 332.00 ± 39.72	a 401.17 ± 7.27	b 143.82 ± 5.48	ab 172.21 ± 16.53	a 400.89 ± 71.79

- Values were expressed as means ± SD.
- Values with different letters are significantly different ($p \leq 0.05$).
- Number of animals 5 rats/ group.

Each of the body weights, testicular and prostatic weights was not change significantly in cadmium-treated group with or without vitamin E treatment as compared with the control. On the other hand, treatment of animals with cadmium produced significant decrease ($P \leq 0.05$) in epididymis and seminal vesicle

weights when compared with the control. Vitamin E administration with cadmium did not improve the results.

Treatment of rats with cadmium produced significant changes ($p \leq 0.05$) in sperm characters as shown in figure (1). Oral administration of rats with cadmium caused significant decrease ($p \leq 0.05$) in both sperm count and live sperm percent, with a significant increase ($p \leq 0.05$) in the percentage of dead and morphologically abnormal sperms as compared with the control group. Treatment of rats with vitamin E after 30 days of Cadmium administration (group 3) caused no changes in sperm count with a significant improvement ($p \leq 0.05$) in live sperm percentage and a significant decrease ($p \leq 0.05$) in the percentage of dead and morphologically abnormal sperms as compared with cadmium-treated groups. Since, treatment with vitamin E returns the values of the live and dead sperms percent to original control values, although the percentage of sperm abnormality was not coming back to control value.
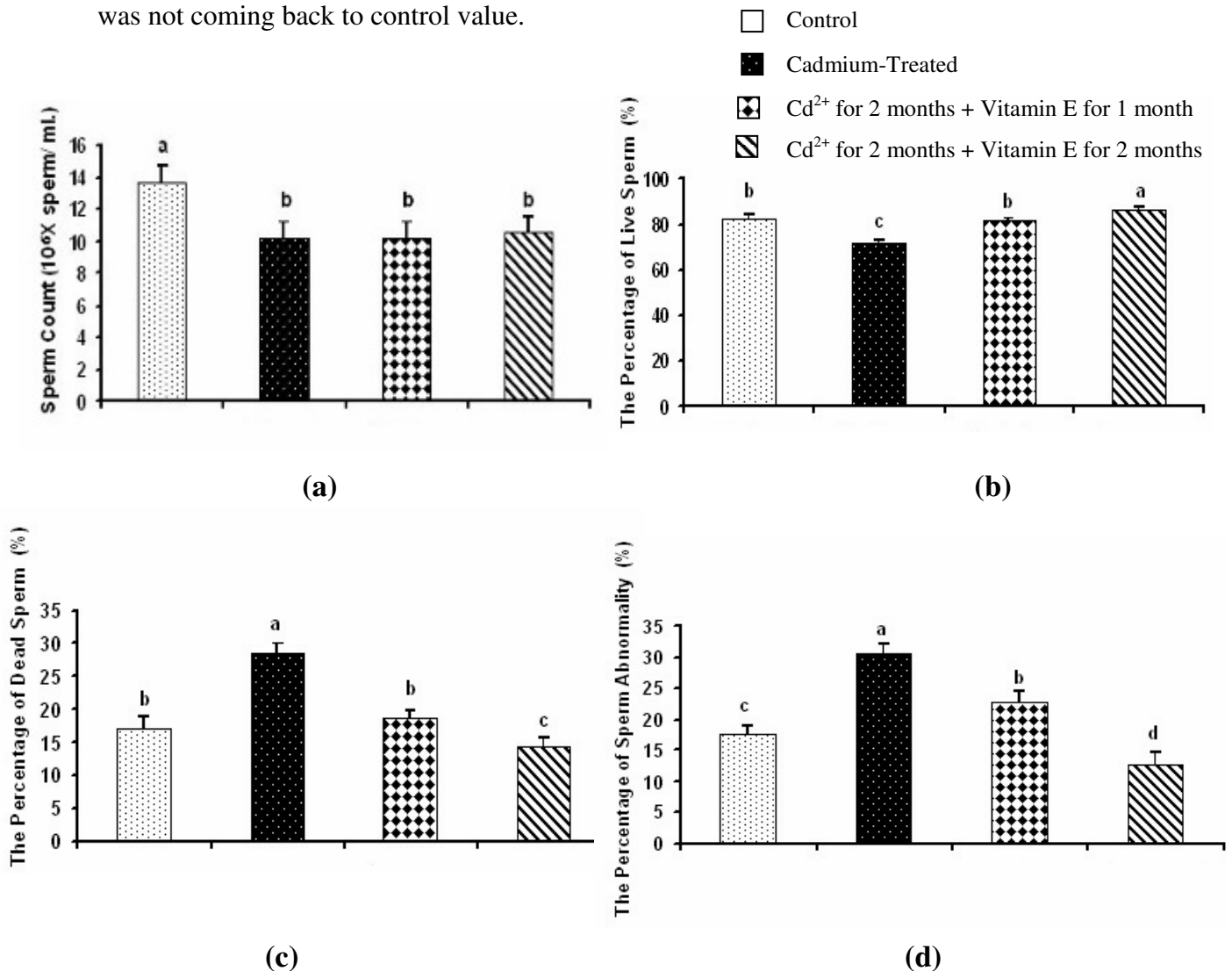

(a)   **(b)**

(c)   **(d)**

Figure1: Effect of 60 days oral administration of cadmium (Cd^{2+}) and vitamin E on sperm characters including sperm count (a), the percentage of live sperm (b), the percentage of dead sperm (c), and the percentage of sperm abnormality (d) in albino male rats. Data are means ± SD. The different letters on histograms indicate significant difference at $p \leq 0.05$.

As compared to cadmium-exposed group, treatment of rats with vitamin E from the first day of cadmium exposure produced no changes in sperm count with a significant increase ($p \leq 0.05$) in sperm live percent and a significant decrease ($p \leq 0.05$) in the percentage of dead and morphologically abnormal sperms. Thus, vitamin E administration to these groups acts to return the values of live, dead and morphologically abnormal sperms percent to original control values. And by this way try to prevent an oxidative damage produced by cadmium in most parameters involved sperm characteristic.

DISCUSSION

Cadmium is the main cause of oxidative stress markers and that stress could be considered in the pathogenesis of cadmium-related diseases (3). The association of cadmium with testicular diseases may be related to the ability of cadmium to induce oxidative stress, which could in turn cause oxidative damage to DNA (18).

The results of the present study demonstrated that cadmium-induced oxidative stress resulted in a significant decrease ($p \leq 0.05$) in epididymal and seminal vesicle weights when compared with the control although other parameters were significantly unchanged. These results can be compared with those obtained by other investigators (19), who observed a significant decrease ($p \leq 0.05$) in weights of testes and epididymis due to cadmium chloride administration to rats at a dose of 5 mg/kg body weight. Moreover, many reports noticed that oral administration of cadmium at a high dose (50 or 100 ppm) to Sprague-Dawley rats induce marked testicular necrosis (20). Similar results about body weight seem to agree with those obtained by other investigators (21) who noticed no change in body weight after oral administration of cadmium (25 mg/L in drinking water). This can be attributed to low dose of cadmium used in the present study. On the other hand, vitamin E given along with (group 3) or after cadmium treatment (group 4) failed to increase testticular weight significantly. Opposite results has been reported by other investigators (22), suggested a significant increase (improvement) in testicular weight when cadmium administered to male rats after vitamin E treatment for five weeks, although the administration of vitamin E to rats in the present study was given at the same time with cadmium.

In the present study, oral administration with cadmium caused significant decrease ($p \leq 0.05$) in both sperm count and live sperm percentage, with an associated significant increase ($p \leq 0.05$) in the percentage of dead and abnormal sperms as compared with the control group.

Cadmium is known to exert toxic effects on multiple organs, including the testes (22). Spermatogenesis is a sophisticated and complex differentiation process (23). Degeneration of spermatogonia is an integral and important part of normal spermatogenesis (23). In the rat, available evidence suggests that cell loss in the spermatogonial stages probably exceeds 75% (24). However, spermatogonial degeneration can result from exposure to some predisposing factors (heat and radiation) (25), and toxic chemicals such as cadmium (22), that may cause irreversible testicular damage and lead to permanent spermatogonia loss and infertility.

Spermatozoa, like all cells living in aerobic conditions, constantly face the oxygen (O_2) paradox: O_2 is required to support life, but its metabolites such as

reactive oxygen species (ROS) can modify cell functions, endanger cell survival, or both (26). Hence, ROS must be continuously inactivated to keep only a small amount necessary to maintain normal cell function. It is not surprising that a battery of different antioxidants is available to protect spermatozoa against oxidants (27). Gomez *et al.,* (28) have indicated that the link between poor semen quality and increased ROS generation lies in the presence of excess residual cytoplasm (cytoplasmic droplet). Under these circumstances, the spermatozoa that are released during spermiation are believed to be immature and functionally defective (29). Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanisms that may be mediated by the cytosolic enzyme glucose-6- phosphate dehydrogenase (G6PD) (30).

Recent studies by Ollero *et al.,* (31) and Gil-Guzman *et al.,* (32) have shown that levels of ROS production in semen were negatively correlated with the percentage of normal sperm forms as determined by World Health Organization (33) and by Kruger's strict criteria (34). These support the results of the present study which indicate that there was a relationship between oxidative stress induced by cadmium and increased the percentage of morphologically abnormal sperms.

Seminal oxidative stress (OS) develops as a result of an imbalance between ROS generating and scavenging activities (35). Spermatozoa are particularly susceptible to OS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs); (36) and their cytoplasm contains low concentrations of scavenging enzymes (37). However, by concomitant administration of cadmium and vitamin E, these changes are diminished and tended to reach control values except for the sperm count.

Generally, antioxidant defense mechanisms include three levels of protection: 1) prevention, 2) interception (chain breaking), and 3) repair (27). Vitamin E is a chain-breaking antioxidant that exists in cell membranes (38). It eliminates lipid peroxyl (RO•) and alkoxyl (ROO•) radicals, suppresses the chain reaction of LPO and promotes the production of scavenger antioxidant enzymes (39). On this basis, vitamin E can not be able to repair the damage caused by oxidants completely when administered 30 days after cadmium treatment (group 3). Furthermore, and unfortunately, spermatozoa are unable to repair the damage induced by OS because they lack the cytoplasmic enzyme systems that are required to accomplish this repair. This is one of the features that make spermatozoa unique in their susceptibility to oxidative insult (40, 41). Thus, Vitamin E in group 4 (used as a protective antioxidant) enables to improve most of the sperm characters more than that used in group 3 (as therapeutic antioxidant).

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