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The Effects of Tocopherol Extraction from Lepidium Sativum Seeds on the Histology of Testis, Epididymis, and Seminal Vesicles of Adult Male Rabbits.

Nada Saad Naji [1]* Fakhir Magtoof Shumran [2]

1. Department of General Sciences, College of Basic Education, University of Babylon, PO box 4, Iraq
2. College of pharmacy, University of Babylon, PO box 4, Iraq

* E-mail of the corresponding author: Dr_n_saad_Altaae@yahoo.com

Abstract
Garden cress or "hab arachad" seeds are considered one of the popular medicinal herbs used in Arabian countries. Garden cress meal (*Lepidium sativum*) is a by-product remaining after the extraction of the oil from seeds. The decline in male reproductive health and fertility in the last 30 years has been linked to environmental toxicants. The present study was designed to investigate the effects of tocopherol extraction of *Lepidium sativum* seeds on histological changes in testis, epididymis, and seminal vesicle tissues of adult male rabbits. Then the ability of the extract to induce changes in the morphology of rabbits organs compared to normal groups was determined by histological study using light microscopy. The results have been showed that oral tocopherol supplementation can improve histoarchitecture of rabbit testis. This suggests that tocopherol extraction could be used to enhance rabbit fertility.
Keywords: *lepidium sativum*, seeds, tocopherol, soxhlet device, rabbits, sperm parameters.

1. Introduction

Many natural dietary agents, including vegetables, fruits, herbs, and spices have been used in traditional medicines, as non-conventional treatments, for thousands of years, but without sufficient scientific proofs. If effective, natural agents might lead to the development of natural and novel drugs with low or no side effects (Mahassni & Al-Reemi 2013). *Lepidium sativum* (family Brassicaceae) locally known as 'garden cress' has been used widely in different parts of the world for its wide therapeutic application, a number of recent studies pointed out the traditional uses of *Lepidium sativum* seeds extract in controlling many clinical problems (Gill & MacLeod 1980), and Ahmed *et al.* (2013) revealed that *Lepidium sativum* seeds with high nutritional value can be exploited as a functional food ingredient. Garden cress is usually cultivated for its leaves, which are used in salad, sandwiches etc (Lefroy Valley carries 2013). The leaves and seedpods have a peppery taste, and has been reported to have enormous biological activities (Sarikami & Yanmaz 2011). It is documented to possess, tocopherol, phenolic compounds, nitrogen compounds, terpenoids, and some other endogenous metabolites, which are rich in antioxidant activity (Muanda *et al.* 2011). Vitamin E was first isolated from green leafy vegetables by Herbert Evans and Katherine Bishop, two prominent researchers from Berkeley and described as a fertility factor in 1922, then was named tocopherol in 1924 and synthesized in 1938 (Sen *et al.* 2007). Moreover, several studies demonstrated that supplementation of vitamin E, the major biological form of vitamin E, could reduce risks of infertility, neurological disorders, inflammation, cardiovascular diseases, diabetes, and certain types of cancers in humans (Traber & Sies 1996, Tucker & Townsend 2005). Vitamin E refers to a family of tocopherols and tocotrienols, Vitamin E cannot be synthesized by humans and must be obtained from the diet with an abundant source found in vegetable oil, nuts, and egg yolks (Ni & Yeh 2007). To date, there are approximately 100 publications on this topic, which highlight the beneficial effects of this antioxidant on viability, membrane integrity and motility of spermatozoa of different species. The protective effects of tocopherol against oxidative damage of sperm cells become even more significant when hygienic conditions are poorly controlled, as they frequently occur in field. Such conditions are associated with increased incidence of infections/inflammations of reproductive apparatus. Therefore, the objective of the present study was conducted to evaluate the histological effects in male rabbits reproductive organs supplement with tocopherol extract of *Lepidium sativum* seeds.

2. Materials and methods

2.1 Preparation of extracts

Garden cress seeds (*Lepidium sativum* L.) were obtained from the local market in Hilla City, Iraq. The seeds were cleaned and rendered free of dust, then stored in polyethylene bags in the refrigerator until use. For tocopherol extraction , garden cress seeds were crushed, using a household mill (Braun, Germany), and then 5 gram sample was extracted with 25mL of (85% hexane : 15% ethyle acetate) for 24 hour by Soxhlet method. The extract was filtered out and evaporated to dryness by oven at 45C for 24 hour (Harborn 1984).

2.2 Experimental animals

Twenty (20) new Zealand white male rabbits aged 4 months and averagely weighing 1.513 gm were balanced for weight and put in cage under control of water, diet, light duration (12 hour light-12hour dark). These animals were divided into 4 groups (5 animals for each group), control group was treated orally with corn oil, and experimental groups were treated orally with 32, 64, and 96 mg/kg body weight of tocopherol extraction, daily for 50 days.

2.3 Histological examination

At the end of the experiment, both control and experimental rabbits were sacrificed after 50 days of treatment and left tissues were taken and fixed in 10% neutral formalin solution for histological assessment. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned and stained with haematoxylin-eosin, and slides were prepared from the tissues , then microscopically examined.

3. Results

The micrograph of the testes of male rabbits on the control (corn oil) is shown in figure 1A while that of the treated group supplemented with 32, 64, 96 mg/kg of tocopherol extraction is presented in figure 1B, C, and D respectively. No histological changes or alterations were observed in the testes of groups treated with 32, 64 mg/kg that showed normal arrangement of the seminiferous tubules with normal germinal epithelium as well as developing and mature spermatozoa. The lumen was seen packed with spermatids and spermatozoa, while the group treated with 96 mg/kg showed observable increase in sperm concentration which represented by the increase of the population of the sperm cells in the lumen of the seminiferous tubules (fig. 1D). Also the micrographs of the seminal vesicles and epididymis of male rabbits treated with 0, 32, 64, and 96 mg/kg of tocopherol extraction have been revealed normal Histological changes as shown in figure 2 and 3.

4. Discussion

Vitamin E supplementation has become a common procedure to promote growth and health and improve the qualitative characteristics of farm animals. It has been demonstrated to be an efficient strategy for improving their reproductive function. Germ cells are particularly vulnerable to oxidative damage and may thus require additional antioxidant production (Castellini *et al.* 2007). In general, our results have been shown that vitamin E has no significant effects on the histology of rabbit testis (except the 96 mg/kg), seminal vesicles, and epididymis. The reason for this is attributed to no pathological or undesired histological changes because the experiment performed on normal model to show the effects of vitamin E, while the other comparative studies on vitamin E were showed the significant effect on pathological and undesired histological changes (Sahinturk *et al.* 2007, Ranade *et al.* 2011, Amao *et al.* 2012). The 96 mg/kg dose showed observable increase in sperm concentration, this result may refer to the typical dose of vitamin E and reflect the important role. By mediating the levels of reactive oxygen species/reactive nitrogen species ROS/RNS, vitamin E may modulate the activation and/or expression of redox-sensitive biological response modifiers, and thereby attenuate the cellular events leading to the onset and development of aging and other degenerative disorders (Chow & Chow-Johnson 2013). In addition vitamin E is quite an effective antioxidant which protects rabbit testis against lipid peroxidation (Aydilek *et al.* 2004). Also, the effect of vitamin E at the dose 96 mg/kg may explain that the doses 32 and 64 mg/kg were not sufficiently high to induce observable effects.

5.Conclusion

Administration of tocopherol has no significant histological effects on testes, seminal vesicles, and epididymis of normal rabbits model (except at 96 mg/kg which had observable effects on sperm concentration). These results indicate that vitamin E has a positive role in improving the development of testis in normal male rabbits at high concentration.

References

Ahmed, M.G., Azza, A.M. & Heba, E.E. (2013), "Chemical, Nutritional and Biochemical Studies of Garden Cress Protein Isolate", *NatSci* **1**1(2), 8-13.

Amao, O., Adejumo, D. & Togun, V. (2012), "Testicular Morphometry and Histological Changes in Rabbit Bucks Fed Cottonseed Cake-Based Diets Supplemented with Vitamin E", *Anim Sci Adv* 2(10), 803-812.

Aydilek, N., Aksakal, M. & Karakilcik, A.Z. (2004), "Effects of Testosterone and Vitamin E on the Antioxidant System in Rabbit Testis", *Andrologia* 36(5),277-281.

Castellini, C., Mourvaki, E., Dal Bosco, A. & Galli, F. (2007), "Vitamin E Biochemistry and Function: A Case Study in Male Rabbit", *Reproduction in Domestic Animals* 42(3), 248-256.

Chow, C.K. & Chow-Johnson, H.S. (2013), "Antioxidant Function and Health Implications of Vitamin E", *The Open Nutrition Journal* 7,1-6.

Gill, V. & MacLeod, A.J. (1980), "Studies on Glucosinolate Degradation in *Lepidium Sativum* L. Seed Extracts", *Phytochem* 19,1369-1374.

Harborn, J.B. (1984), "Phytochemical methods : A Guide to Modern Techniques of Plants Analysis", 2nd.ed London, New York, Chapman and Hall.

Lefroy Valley carries (LVC). (2013), "Herb Range", Australia/New Zealand, 1300 Lefroy www.Lefroyvalley.Comout .

Mahassni, S. & Al-Reemi, R.M. (2013), "Apoptosis and Necrosis of Human Breast Cancer Cells by an Aqueous Extract of Garden Cress (*Lepidium Sativum*) Seeds", *Saudi Journal of Biological Sciences* **12** (2),1-9.

Muanda, F.N., Bouayed, J., Djilani, A., Yao, C., Soulimani, R. & Dicko, A. (2011), "Chemical Composition and, Cellular Evaluation of the Antioxidant Activity of *Desmodium adscendens* Leaves", *Evidence-Based Complementary and Alternative Medicine*, 1-9.

Ni, J. & Yeh, S. (2007), "The Roles of Vitamin E and its Analogues in Prostate Cancer", *Vitamins & Hormones* **76**, 493- 518.

Ranade, A.V., Tripathi, Y., Rajalakshmi, R., Vinodini, N.A., Soubhagya, R.N., Nayanatara, A.K., Rekha, D.K. & Kumari, M. (2011), "Effect of Vitamin E Administration on Histopathological Changes in Rat Testes Following Torsion and Detorsion", *Singapore Med* **52**(10), 742 -746.

Sahinturk, V., Guclu, C. & Baycu, C. (2007), "Protective Effects of Vitamin E on Ethane Dimethane Sulfonate-Induced Testicular Toxicity in Rats", *Asian J Androl* **9** (1), 117–124.

Sarikami, G. & Yanmaz, R. (2011), "Effects of Cultivar and Developmental Stage on Glucosinolates in Garden Cress (*Lepidium Sativum* L.) ", *Medicinal Plants Research* **5**(17), 4388-4392.

Sen, C.K., Khanna, S., Rink, C. & Roy, S. (2007), "Tocotrienols: the Emerging Face of Natural Vitamin E", *Vitam Horm* **76**, 203-61.

Traber, M. & Sies, H. (1996), "Vitamin E in Humans: Demand and Delivery", *Annu. Rev.Nutr* 16,321 347.

Tucker, J.M. & Townsend, D.M. (2005), "Alpha-Tocopherol: Roles in Prevention and Therapy of Human Disease", *Biomed. Pharmacother* 59, 380–387.

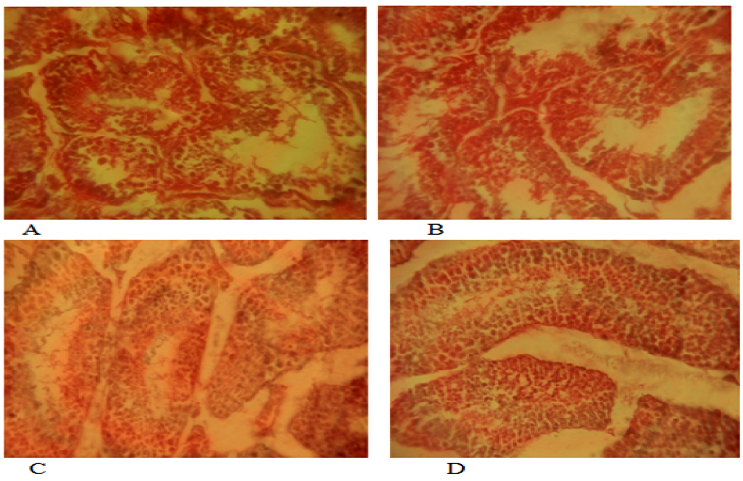

Figure 1. Histological Sections of the Testis of Rabbits Stained with Haematoxylin and Eosin. A: Control Group Showing Normal Histology. B, C: 32, 64 mg/kg (Respectively) Treated Group Showing Normal Histology. D: 96 mg/kg Treated Group Showing Observable Increase in Sperm Concentration

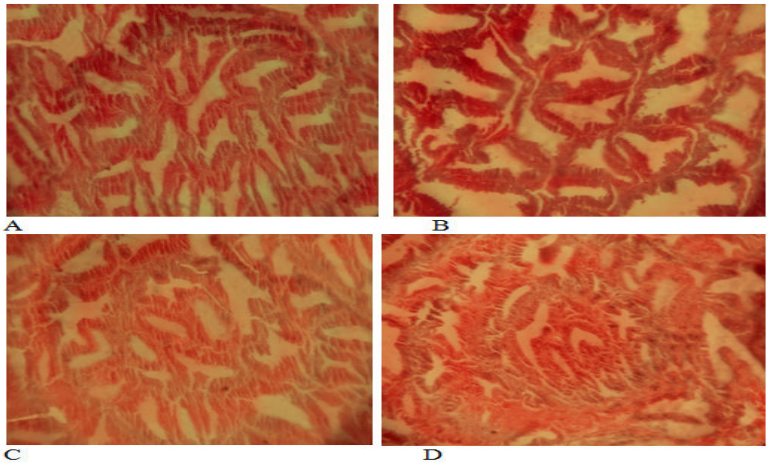

Figure 2. Histological Sections of the Seminal Vesicles of Rabbits. A: Control Group Showing Normal Histology. B, C, and D: 32, 64, and 96 mg/kg (Respectively) Treated Groups Showing Normal Histology

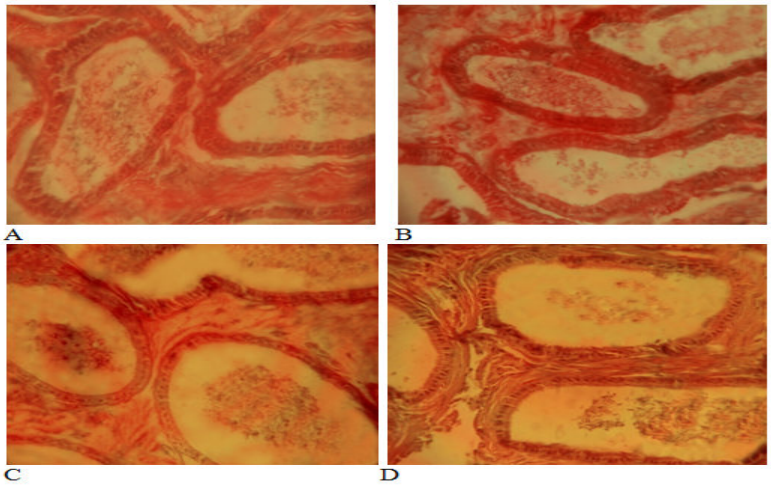

Figure 3. Histological Sections of the Epididymis of Rabbits. A: Control Group Showing Normal Histology. B, C, and D: 32, 64, and 96 mg/kg (Respectively) Treated Groups Showing Normal Histology.