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Effect of Tocopherol Extraction of *Lepidium Sativum* Seeds in Sperm Parameters of White Male Rabbits

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Abstract
The objective of this research was to investigate the effect of tocopherol extraction of *Lepidium sativum* seeds on the fertility of adult male rabbit. The *In Vivo* study included the estimation of the Medium Effect Dose (MED_{50}) for tocopherol extraction by using Dose-Response Curve. The mean of MED_{50} was 33.6 mg/kg body weight (b.w.) of tocopherol. The results showed a significant increase ($P < 0.05$) in testicular sperm concentration, epididymus sperm concentration and in the sperm count per gm of the testis, sperm motility percent, grade activity, sperm viability percent. While it was found a decrease in abnormal sperm morphology percent of epididymis caudal at MED_{50} of tocopherol. Our results reveal that due to tocopherol administration; male rabbits fertility increases, thus being useful to any animal, mammals as well as human being.
Keywords: Lepidium sativum, seeds, tocopherol, rabbits, sperm parameters

1.Introduction

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, and Garden cress meal is a by-product remaining after the extraction of the oil from seeds. It 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 have 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). Rashad seeds contain two sets of fat-soluble compounds, and represent the first group of tocopherols, which consists of (21ppm) alpha-tocopherol and (1422 ppm) gamma-tocopherol and (35ppm) Sigma-tocopherol, The second group are tocotrienols (Moser *et al.* 2009). 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). Although vitamin E has been known as an essential nutrient for reproduction since 1922, we far from understand the mechanisms of its physiological functions (Brigelius-Flohe & Traber 1999). 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.

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 used. For tocopherol extraction, garden cress seeds were crushed, by using a household mill (Braun, Germany), and then 5 g 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 45^0C for 24 hour (Harborn 1984).

2.2Experimental animals

Twenty (20) New Zealand White male rabbits aged 4 months and averagely weighing 1.513 gram and put in cage under control of water, diet, light duration (12hour 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 b.w. of tocopherol extraction and for 50 days daily with these concentration. The animals were seduced after a period end of experience with chloroform then the epididymis caudal was excised to calculate the rate of sperm concentration in epididymis caudal, sperm motility percent, and grade activity, and find MED_{50} for each parameter, and then calculating the average of the above doses affecting. Effective dose of extract was used after its appointment in the first experiment to know its impact on

reproductive efficiency of rabbits, using 10 male rabbit, and these animals were divided into 2 groups, and by 5 animals per group and treated as described below:

- Group A: control group, treated orally with corn oil for 50 days.
- Group B: tocopherol group, treated orally with 33.6 mg/kg b.w. of extract for 50 days.

The animals have been weighed after the expiration of the effective dosage using the balance, and they were seduced after a period end of experience with chloroform and all of the left testicle and left epididymis were used to study of sperm parameters.

2.3 Statistical analysis

Results were presented as Mean ± S.E. Statistical analysis was done using SPSS and was conducted using one way ANOVA to compare the means, F-test, T-test, correlation coefficient, and use the Least Significant Difference (LSD) in the comparison between the results and also regression analysis and regression coefficient. Different letters was considered significant ($P<0.05$) (Randolph & Ciminera 1985; AL-Rawi 2000).

3. Results

In Vivo Experiments, the treatment of animals with 32 and 64 and 96 mg/kg b.w. of tocopherol extraction led to get a significant increase ($P<0.05$) in each of sperm concentration of epididymis caudal and sperm motility percent for the treated groups, compared with the control group, with no significant difference ($P>0.05$) between groups. It has been observed a significant increase ($P<0.05$) in sperm motility percent with progressive movement of the epididymis caudal for the group treated with 32 mg/kg b.w., compared with the control group and the groups treated with 64 and 96 mg/kg b.w., and insignificant differences ($P>0.05$) between the treatment group at 64 and 96 mg/kg b.w. (Fig.1,2 and 3).

Figures 4, 5 and 6 show a positive linear relationship between escalating doses of tocopherols extract and sperm concentration, sperm motility percent, and sperm motility percent with progressive movement of the epididymis caudal where the correlation coefficient were $r = 0.756$, 0.559, and 0.293; and the regression equation linear standard as follows: $y = 103.24 + 0.995 (x)$, $y = 85.36 + 0.108 (x)$, $y = 71.72 + 0.082 (x)$ of the above parameters, respectively.

The results revealed a significant increase in the three parameters of the epididymis caudal, so the values of MED50 were 26.39, 16.019, and 58.382 mg/kg b.w. of tocopherols extract of the three criteria respectively. The average was calculated for these values in order to be used in a subsequent experiment. Accordingly, the value is equal to 33.6 mg/kg b.w. of tocopherols extract as shown in the table (1).

4. Discussion

The results showed that the use of tocopherol extract of Rashad seeds led to a significant increase in the rate of sperm concentration in epididymis caudal. Because the tocopherol play a vital role for the activity of the body (Jaiswal *et al.* 2004), and its fat-soluble antioxidant and kept the body from various chemical reactions of free radicals, which poison the cells (Braun & Cohen 2007). The results of the present research agree with the findings of the Frank *et al*. (2003) in their study on male rats treated with roots extract of *Lepidium meyenii* plant, which led to improved sexual function, and activate the process of sperm formation in Mitotic stages. And increase sperm production by 85% and sperm motility by 5% in humans (Chung *et al.* 2005). The researcher (Mahaneem *et al.* 2011**)** has pointed out that the increase in the sperm concentration of rats treated orally at 1.2 g/kg b.w. of honey for 28 days, returning to the presence of some antioxidants, including phenols, flavonoids, vitamin A, E and catalase enzyme. Our results showed that the dosage of white male rabbits with influential concentration of tocopherol extract; led to a significant increase in sperm motility percent, grade activity, and sperm viability percent of epididymis caudal. The results of the research agreed with the results of (Gonzalez *et al.* 2001**)** at 9 men dosage with 1500 and 3000 mg/kg/day from *Lepidium meyenii* roots for 4 months, since it was found increase in the sperm concentration and its activity. Several researchers noted that Black Maca of *Lepidium meyenii*, more efficient in increasing of sperm concentration and sperm motility percent, compared to the rest of other types (red root and yellow root) in male rats after 42 days of treatment, possibly due to the influence of the Black Maca along the eighth stage of seminiferous epithelial tubules (Gonzales *et al.* 2006; Yucra *et al.* 2006). The decrease in abnormal sperm morphology percent of rabbits epididymus caudal treated with effect dose of tocopherol extract in the current study, was similar to another study (Christie 2010), which indicated that tocopherol, may protect sperm through its function as antioxidant of body tissues, by a network of cellular anti-oxidation defenses, where inhibits alpha-tocopherol enzyme protein Kinase, and thus reduces the reactive oxygen species. The researcher (Audet *et al.* 2004**)** showed that feeding piglets with vitamins, including vitamin E led to an increase in testosterone concentration in the blood plasma of animals treated after puberty, sperm production, and sperm motility percent and no change in abnormal sperm morphology percent compared to the control groups. Vitamin E acts with other antioxidants, including vitamin C, glutathione, selenium, and beta-carotene; in prevent lipid peroxidation, and reduce DNA damage (Meletis *et al.*

2006; Brink 2010). The tocopherol working in scavenging free radicals, and it concludes the body from free radicals, which cause DNA damage (Naseem *et al.* 2007; Diggs 2009). The DNA damage by free radicals caused to descendant distortions due to contain sperm membranes a high concentration of unsaturated fatty acids, which are more susceptible to damage with types Reactive Oxygen Species (Sancheza *et al.* 2009). 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 require additional antioxidant protection (Castellini *et al.* 2007). Also vitamin E is quite an effective antioxidant which protects rabbit testis against lipid peroxidation (Aydilek *et al.* 2004).

5. Conclusion

Under the light of this research, it is concluded that tocopherol extraction of *Lepidium sativum* seeds is the most important fat-soluble antioxidant .Our results revealed a significant increase in testicular sperm concentration, epididymus sperm concentration and in the sperm count per gm of the testis, sperm motility percent, grade activity, sperm viability percent, and decreased in the abnormal sperm morphology percent of epididymus caudal. This suggests that tocopherol extraction of garden cress seeds (*Lepidium sativum* L.) could be used to enhance human fertility.

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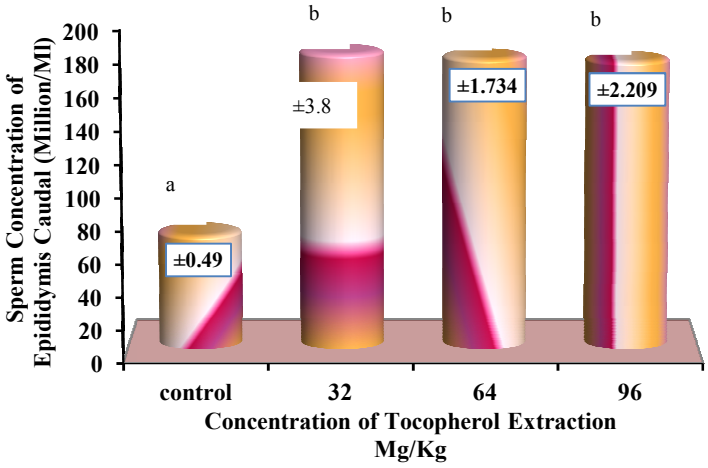

Figure 1. Impact of Escalating Doses of Tocopherol Extract in the Rate of Sperm Concentration in the Epididymis Caudal (million/ml) of Male Rabbits

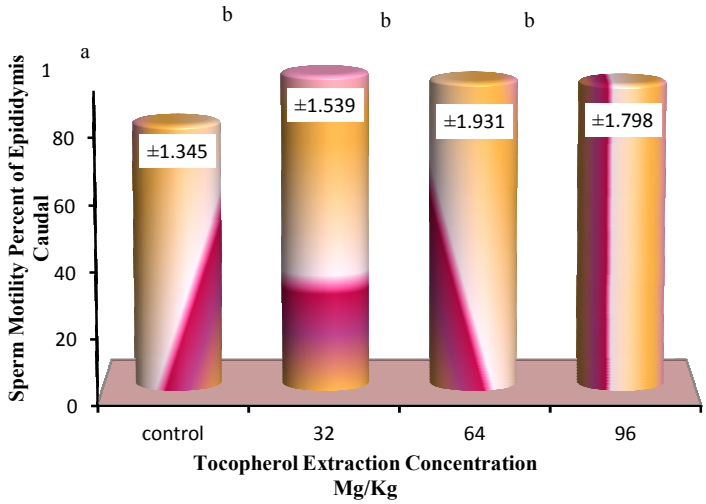

Figure 2. Impact of Escalating Doses of Tocopherol Extract in the Sperm Motility Percent in the Epididymis Caudal (Million/Ml) of Male Rabbits

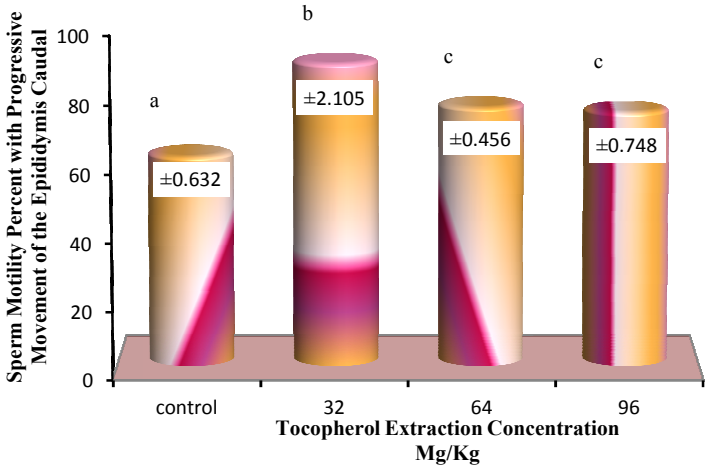

Figure 3. Impact of Escalating Doses of Tocopherol Extract in the Sperm Motility Percent with Progressive Movement in the Epididymis Caudal (Million/Ml) of Male Rabbits

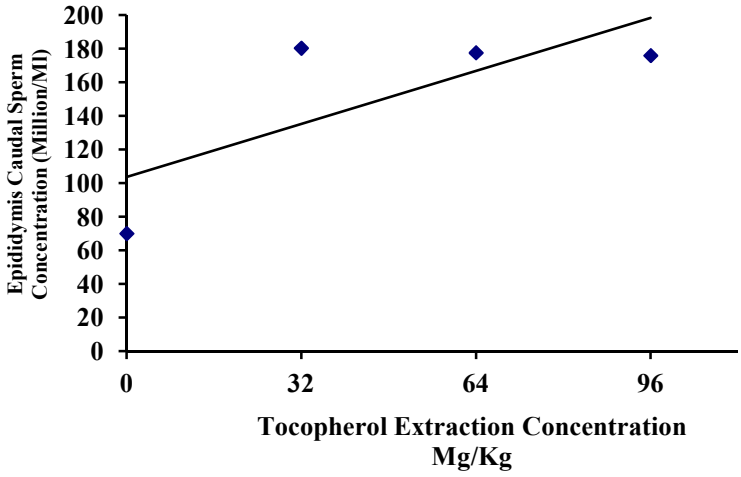

Figure 4. Variation of Sperm Concentration Rate with Escalating Doses of Tocopherol Extract in Male Rabbits

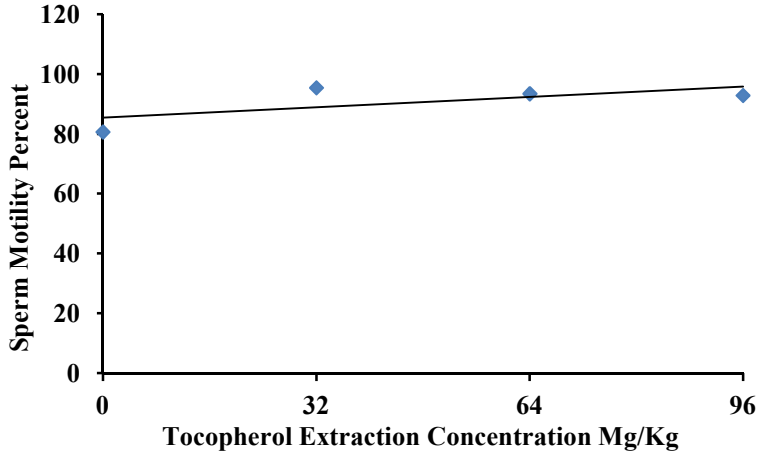

Figure 5. Variation of Sperm Motility Percent with Escalating Doses of Tocopherol Extract in Male Rabbits

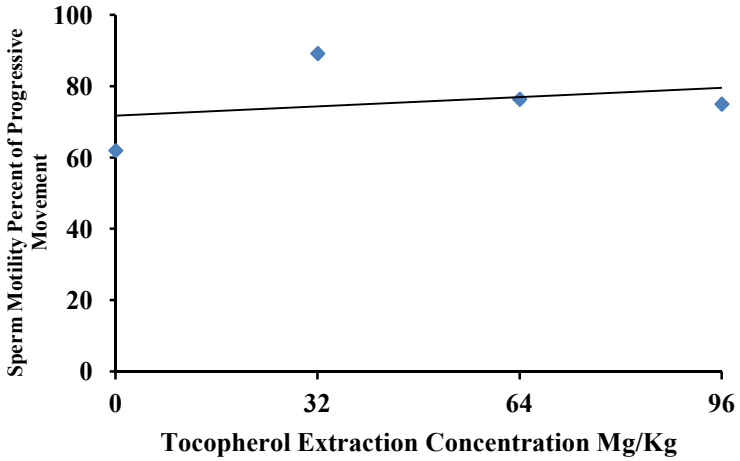

Figure 6. Variation of Sperm Motility Percent of Progressive Movement with Escalating Doses of Tocopherols Extract in Male Rabbits

Table 1. Median Effective Dose 33.6 Mg/Kg B.W. of Tocopherol Extract in Sperm Parameters of the Testis and Epididymis Caudal of White Male Rabbits after Oral Dosing for 50 Days

Groups / Sperm Parameters	Control	Effect Dose 33.6Mg/Kg/Day of Tocopherol Extraction
Testicular Sperm Concentration (Million/Ml)	a 32.80 ± 0.769	b 81.00 ± 2.173
Epididymis Caudal Sperm Concentration (Million/Ml)	a 67.80 ± 0.769	b 116.00 ± 2.173
Sperm Count Rate Per Gm of the Testis	a 13.94 ± 0.407	b 30.48 ± 0.703
Sperm Motility Percent of Epididymis Caudal	a 76.40 ± 1.757	b 95.60 ± 1.117
Sperm Motility Percent with Progressive Movement (a+b) of the Epididymis Caudal	a 60.00 ± 0.633	b 72.40 ± 1.152
Sperm Motility Percent with Progressive Movement (c) of the Epididymis Caudal	a 16.00 ± 0.632	b 23.00 ± 1.233
Sperm Motility Percent with Progressive Movement (d) of the Epididymis Caudal	a 22.40 ± 0.607	b 4.80 ± 0.335
Abnormal Sperm Morphology Percent of Epididymis Caudal	a 27.40 ± 0.727	b 20.80 ± 1.730
Sperm Viability Percent of Epididymis Caudal	a 76.40 ± 2.273	b 85.00 ± 1.233

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