

## Changes in Sperm Parameters of Adult Male Rabbits by Phenol Extract of *Lepidium Sativum* Seeds

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### Abstract

This research included the impact of phenol extract of *Lepidium sativum* seeds in sperm parameters of adult male rabbit. The *In Vivo* study included the estimation of the Medium Effect Dose (MED<sub>50</sub>) for phenol extraction by using Dose-Response Curve. The mean of MED<sub>50</sub> was 36.1 mg /kg body weight (b.w.) of phenol. 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, and abnormal sperm morphology percent of epididymis caudal at MED<sub>50</sub> of phenol. The results reveal that supplement with low doses of phenol could be enhance rabbit fertility.

**Keywords:** *Lepidium sativum*, seeds, phenol, soxhlet device, rabbits, sperm parameters

### 1. Introduction

The *Lepidium sativum* (family Brassicaceae) is a native shrub (Gill & MacLeod 1980). It is usually cultivated for its leaves, which are used in salad, sandwiches etc. (Lefroy Valley carries (LVC) 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, phenol compounds, tocopherol, nitrogen compounds, terpenoids, and some other endogenous metabolites, which are rich in antioxidant activity (Muanda *et al.* 2011). and Ahmed *et al.* (2013) revealed that *Lepidium sativum* seeds with high nutritional value can be exploited as a functional food ingredient. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress and a variety of free radical scavenging antioxidants are found in dietary sources like fruits, vegetables and tea (Souri *et al.* 2008). Phenolic compounds are a large group of phytochemicals widespread in the plant kingdom. Depending on their structure they can be classified into simple phenols, phenolic acids, hydroxyl cinnamic acid derivatives and flavonoids (Cartea *et al.* 2012). 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 phenol extraction, garden cress seeds were crushed, by using a household mill (Braun, Germany), and then 1 g sample was extracted with 25mL of (80% methanol : 10% Distilled water) in a water bath at 70°C for 50 minutes. The extract was filtered out and evaporated to dryness by oven at 45°C for 24 hour (Harborn 1984).

#### 2.2 Experimental 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 distilled water and experimental groups were treated orally with 32, 64, and 96 mg/kg b.w. of phenol extract 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<sub>50</sub> 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 distilled water for 50 days.
- Group B: phenol 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  $\pm$  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 were 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 phenol extract 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. It has been observed a significant increase ( $P < 0.05$ ) in sperm motility percent with progressive movement of the epididymis caudal for the groups treated with 64 and 96 mg/kg b.w., compared with the control group and the group treated with 32mg/kg b.w. (Figure 1, 2 and 3). Figures 4,5 and 6 show a positive linear relationship between escalating doses of phenol extract and sperm concentration, sperm motility percent, and sperm motility percent with progressive movement of the epididymis caudal where the correlation coefficient were  $r = 0.865$ ,  $0.789$ , and  $0.874$ ; and the regression equation linear standard as follows:  $y = 78.18 + 0.509(x)$ ,  $y = 76.74 + 0.158(x)$ ,  $y = 54.12 + 0.316(x)$  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 38.958, 27.048, and 42.308 mg/kg b.w. of phenol 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 36.1mg/kg b.w. of phenol extract as shown in the table (1).

### 4. Discussion

The results revealed that phenol extract of *Lepidium sativum* seeds led to a significant increase in the rate of sperm concentration in epididymis caudal, due to its antioxidant and kept the body from various chemical reactions of free radicals, which poison the cells (Dragsted 2003). 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 adult male rabbits with influential concentration of phenol extract; led to a significant increase in sperm motility percent, grade activity, and sperm viability percent of epididymis caudal. This might return to the effect of *Lepidium meyenii* roots, which related to the Rashad plant, and to the association the aromatic compounds, including Isothiocyanates with Aphrodisiac activity, or to link phenol compounds with antioxidants activity, or possibly due to the influence of the Black Maca along the eighth stage of seminiferous epithelial tubules (Gonzales *et al.* 2006; Yucra *et al.* 2008; Singh *et al.* 2011). The increase in abnormal sperm morphology percent of rabbits epididymus caudal treated with effect dose of phenol extract in the current study, may be return to the use of the high concentration of phenol, which may interfere in the process of spermatids formation, which lead to damage in the process of the sperm head configuring, forming abnormal sperm, and perhaps obtained as a result of genetic overlap and cause abnormalities in sperm. This result is consistent with the results of Michalowicz and Duda (2007), due to both of phenolic compounds and Catechol have harmful toxics, and toxicity of phenol may return to the toxic non-specific, relating to Hydrophobicity, the solubility of phenol in the parts of the cell, and thus the possibility of the interaction of these compounds with cell and structures of tissues. Both of Catechol and Hydroquinone Inhibits the effectiveness of the ribonucleotide reductase enzyme, which is involved in the manufacture of DNA (McCue *et al.* 2003). And toxicity of phenols may be back to the roots of Phenoxy and to toxicity resulting from metabolites (Zitka *et al.* 2011). The interact of phenolic acids with proteins lead to change in composition of carbonyl groups and oxidation of thiol groups (Labieniec & Gabryelak 2006). The phenolic compounds own the property of production of hydrogen peroxide in the presence of some metals (Cartea *et al.* 2011), where hydrogen peroxide is a kind of reactive oxygen species, due to its Interaction between the root of the hydroxyl and chains unsaturated fatty acid in the cell membrane; leading what is known as the lipid peroxidation (Vermerris & Nicholson 2006).

### 5. Conclusion

Under the light of this research, it is concluded that phenol extract of *Lepidium sativum* seeds is the most important 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 abnormal sperm morphology percent of epididymus caudal. According to the results it can be concluded that phenol extract improves some parameters of sperm.

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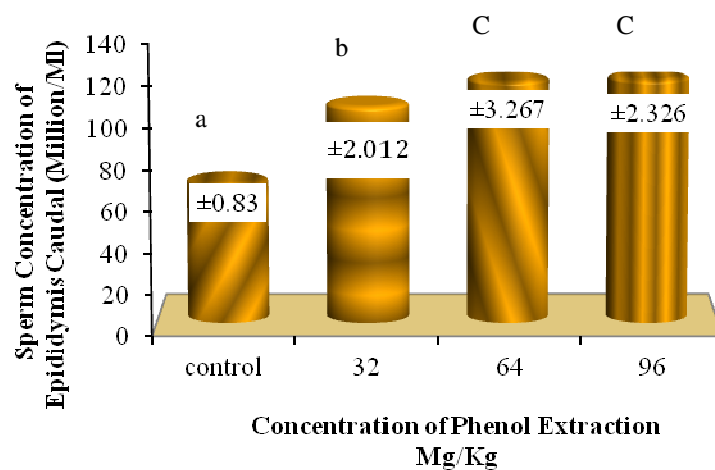


Figure 1. Impact of Escalating Doses of Phenol Extract in the Rate of Sperm Concentration in the Epididymis Caudal (Million/MI) of Male Rabbits

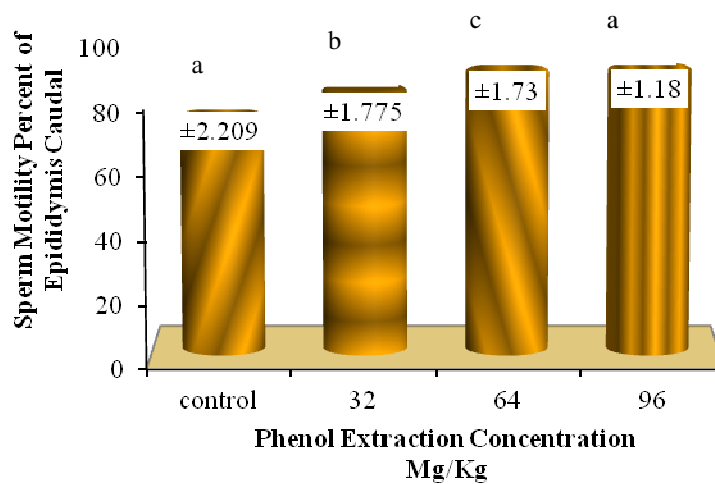


Figure 2. Impact of Escalating Doses of Phenol Extract in the Sperm Motility Percent in the Epididymis Caudal (Million/MI) of Male Rabbits

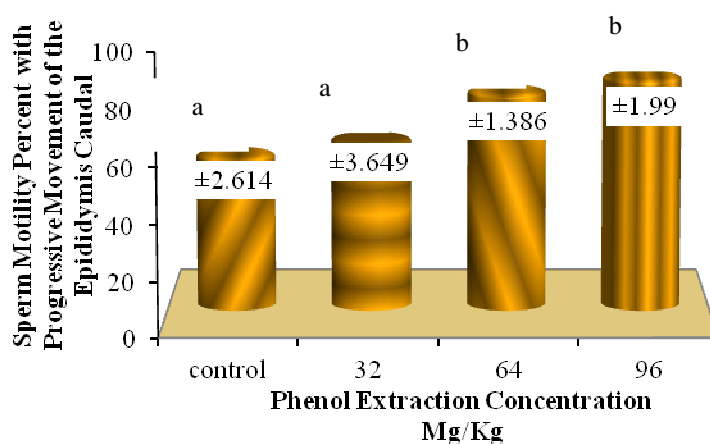


Figure 3. Impact of Escalating Doses of Phenol Extract in the Sperm Motility Percent with Progressive Movement in the Epididymis Caudal (Million/MI) of Male Rabbits

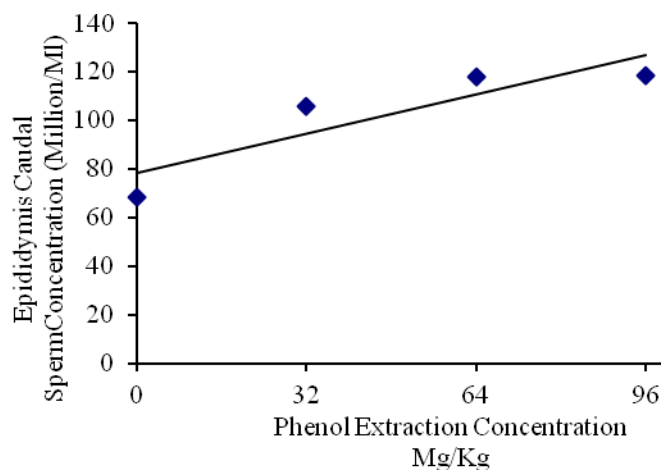


Figure 4. Variation of sperm concentration rate with escalating doses of phenol extract in male rabbits

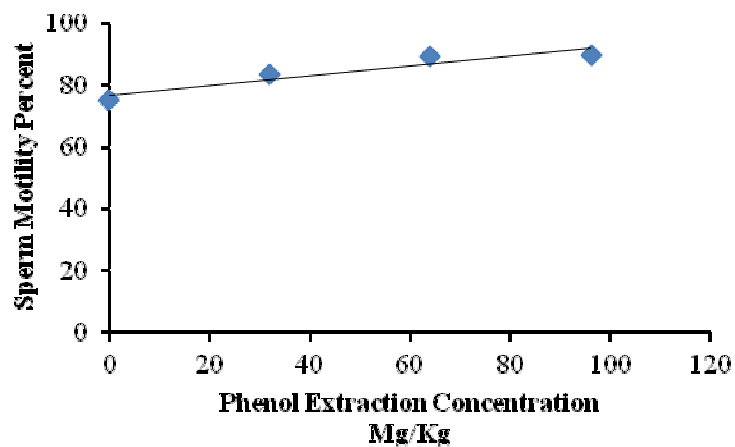


Figure 5. Variation of Sperm Motility Percent with Escalating Doses of Phenol Extract in Male Rabbits

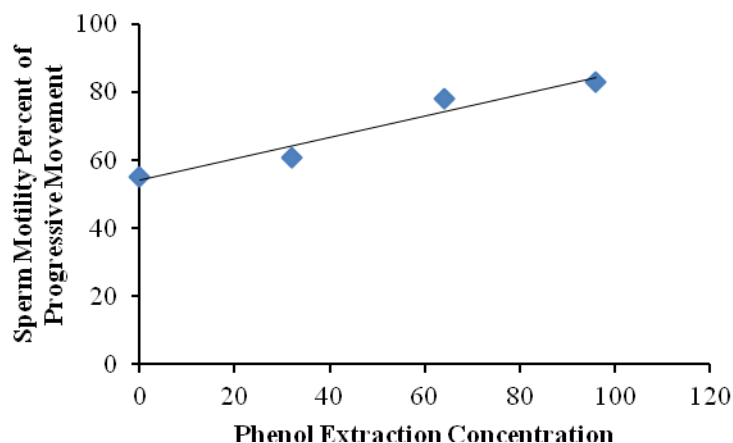


Figure 6. Variation of Sperm Motility Percent of Progressive Movement with Escalating Doses of Phenol Extract in Male Rabbits

Table 1. Median Effective Dose 36.1 Mg/Kg b.w. of Phenol Extract in Sperm Parameters of the Testis and Epididymis Caudal of White Male Rabbits After Oral Dosing for 50 Days

Groups	Control	Effect Dose 36.1 Mg/Kg/Day of Phenol Extraction
<b>Sperm Parameters</b>		
Testicular Sperm Concentration (Million/MI)	a 30.00 ± 1.020	b 79.40 ± 2.273
Epididymis Caudal Sperm Concentration (Million/MI)	a 65.00 ± 1.020	b 114.40 ± 2.020
Sperm Count Rate Per Gm of the Testis	a 12.40 ± 0.439	b 28.80 ± 0.795
Sperm Motility Percent of Epididymis Caudal	a 71.00 ± 2.966	b 86.80 ± 0.593
Sperm Motility Percent with Progressive Movement (a+b) of the Epididymis Caudal	a 52.40 ± 1.513	b 64.60 ± 1.431
Sperm Motility Percent with Progressive Movement (c) of the Epididymis Caudal	a 18.60 ± 0.456	b 22.20 ± 0.522
Sperm Motility Percent with Progressive Movement (d) of the Epididymis Caudal	a 29.00 ± 0.938	b 13.20 ± 0.335
Abnormal Sperm Morphology Percent of Epididymis Caudal	a 24.20 ± 0.593	b 48.20 ± 1.339
Sperm Viability Percent of Epididymis Caudal	a 71.00 ± 0.400	b 83.60 ± 1.345