

## The Role of *Lepidium Sativum* as Free Radical Scavenger in Laboratory Mice

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### Abstract

**Background:** *Lepidium sativum* is a by-product remaining after the oil is extracted from Garden cress seeds. This herb is considered one of the popular medicinal herbs used in Arabian countries and has traditionally been used to control many clinical problems. The present study aims to uncover these seeds' free radical-scavenging properties and their Role in improving the testicular tissues and seminal properties.

**Material and Methods:** Thirty-two sexually mature mice were used, and the treated groups were divided into three groups; the first one (G1) was treated with 0.1 ml of GC seed extraction in a dose of 5mg/kg BW, the second one(G2) was treated with 0.1 ml sodium nitrate as oxidant, and the last treated group (G3) was treated with 0.1 ml of GC seed extraction after one hour of injection of 0.1 ml sodium nitrate.

**Results:** The results showed improvement of the testicular tissue and a significant elevation in sperm viability, in addition to significant enhancements of RBC count and Hb concentration. By its Role as a free radical scavenger, sodium nitrate removed the negative effect on blood parameter values.



## دور بذور الرشاد ككاسح للجذور الحرة في فئران المختبر

### الخلاصة

**الخلفية:** الليبيديوم ساتيفوم هو منتج ثانوي يبقى بعد استخلاص الزيت من بذور حب الرشاد. تعتبر هذه العشبة من الأعشاب الطبية الشعبية المستخدمة في الدول العربية وقد تم استخدامها تقليدياً للسيطرة على العديد من المشاكل السريرية. تهدف الدراسة الحالية إلى الكشف عن خصائص هذه البذور في مكافحة الجذور الحرة ودورها في تحسين أنسجة الخصية وخواص الحيوانات المنوية.

**المواد والطرق:** تم استخدام اثنين وثلاثين فأراً ناضجاً جنسياً، وتم تقسيم المجموعات المعالجة إلى ثلاث مجموعات؛ عولجت المجموعة الأولى ب ٠,١ مل من مستخلص بذور الرشاد بجرعة ٥ ملغم/كغم من وزن الجسم اما المجموعة الثانية عولمت ب ٠,١ مل من نترات الصوديوم كمادة مؤكسدة، بينما المجموعة الثالثة فعولمت ب ٠,١ مل من مستخلص بذور الرشاد بعد ساعة واحدة من حقنها ب ٠,١ مل من نترات الصوديوم

**النتائج:** أظهرت النتائج تحسناً ملحوظاً في أنسجة الخصية وارتفاعاً ملحوظاً في حيوية الحيوانات المنوية، بالإضافة إلى تحسن كبير في عدد كرات الدم الحمراء وتركيز الهيموجلوبين. من خلال دورها ككاسح للجذور الحرة، أزال نترات الصوديوم التأثير السلبي لنترات الصوديوم على قيم معاملات الدم.

## 1. Introduction

*Lepidium sativum* is locally known as 'garden cress' (GC). The plant and its seeds are considered popular medicinal herbs used in many Arabic countries as a good mediator for bone fracture healing in the human skeleton (Dixit Jr *et al.*, 2020).

Several recent studies pointed out the traditional uses of *Lepidium sativum* seed extract in controlling many clinical problems. They were used as anti-asthmatics, antiscorbutics, aperients, diuretics, galactagogues, poultices, and stimulants. The leaves are antiscorbutic, diuretic and stimulant (Ejigu and Endalifer, 2023).

*Lepidium sativum* has been studied pharmacologically for its laxative (3), antibacterial (4), bronchodilatory (5), contraceptive effects (6), and inflammatory bowel disease ( ). The seedlings contain a significant amount of protein, fat, iron, calcium, folic acid, and vitamins A and C. (Doke and Guha, 2014)

The present investigation was undertaken to screen these seeds' free radical scavenging activity and their Role in activating epididymal sperms.

## 2. Material and Methods

The *Lepidium sativum* seeds were purchased from local Mark T. They were cleaned manually to remove dirt, dust, and extraneous mats and were ground with electrical bland r. Oily extracted preparation using hot continuous Soxhlet extraction meth d. ( ). The extract was stored in a dark container in a refrigerator until needed.

Thirty-two sexually mature mice aged 6-7 weeks were used (16 male and 16 female) divided as follows:

1. Control group: 4 males and four females were injected intraperitoneally with normal saline and served as a control group.
2. G1: 4 males and four females were injected intraperitoneally with 0.1 ml of GC seed extraction in a 5mg/kg BW dose.
3. G2: 4 males and four females were injected intraperitoneally with 0.1 ml sodium nitrate as an oxidant.
4. G3: 4 males and four females were injected intraperitoneally with 0.1 ml of GC seed extraction in a dose of 5mg/kg BW after one hour of injecting 0.1 ml sodium nitrate.

The experiment lasted for 21 days, and at the end of the treatment period, the specimens were collected from the mice, including blood for measuring the blood values, epididymis for seminal analysis, and tests for studying the histological changes.

Statistical analysis: experimental data were analyzed using one-way ANOVA

The P-values less than 0.05 were considered statistically significant.

## 3. Results

The injection of GC seed oil extraction significantly elevated RBC count and Hb concentration but had no effect on WBC compared with the control group Table 1.

**Table 1:** Effect of Garden Cress Seeds Extract on Blood Parameters in Mice

Groups	RBC count Cell / cmm*10 <sup>6</sup>	WBC count Cell / cmm*10 <sup>3</sup>	Hb g / dl
<b>G1 Control group</b>	3.16 ±0.02 B	6.56 ± 0.11 A	12.2 ± 0,89 B
<b>G2 Treated with GCS extract</b>	3.34 ± 0.16 A	6.52 ± 0.22 A	13.9 ± 1.04 A

**\*Different Letters Represent Significant Differences at (P≤0.05)**

The blood parameters values (RBC, Hb, and WBC) were suppressed significantly ( $p \leq 0.05$ ) by injection of sodium nitrate as an oxidant. The blood parameters return to their average values by the effect of GC seed oil extraction when injected one hour after sodium.

Nitrate administration Table 2.

**Table 2:** Effect of Garden Cress Seed Extraction as ROS Scavenger

Groups	RBC count cell / cmm*10 <sup>6</sup>	WBC count cell / cmm*10 <sup>3</sup>	Hb g / dl
Control group	6.33 ±0.18 A	3.96 ± 0.29 A	13.42 ± 0.43 A
G3 Treated with sodium nitrate	5.2 ± 0.10 B	2.62 ± 0.1 B	7.1 ± 0.04 B
G4 Treated with GCS extract after 1h of sodium nitrate injection	6.65 ± 0.03 A	3.2 ±0.24 A	11.45 ± 0.63 A

\*Different letters represent significant differences at ( $p \leq 0.05$ )

It seems that the injection of GC seeds oil extraction caused significant ( $p \leq 0.05$ ) elevation in testes weight and sperm count, whereas the dead and deformed sperms number significantly ( $p \leq 0.05$ ) decreased compared to that of normal animals, but it wasn't any effect neither on the viability of the sperms nor the individual and massive movement of the sperms (table 3)

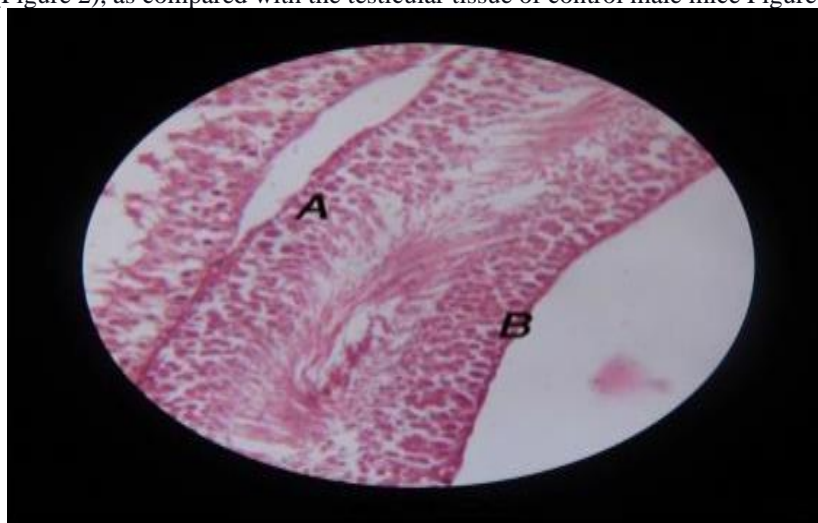
**Table 3:** Seminal Analysis of Male Mice Treated with GC Seed Oil Extraction

Groups	Testes Weight(G)	Viable Sperms	Dead Sperms	Deformed Sperms	Sperms Count	Individual Movement	Massive Movement
Control Group	0.3 ± 0.07 B	91.24 ± 9.1 A	11.5 ± 29.2 A	13.5 ± 8.1 A	194.8 ± 0.54 B	90 ± 0.00 A	90 ± 0.00 A
Treated Group	0.62 ± 0.08 A	92.75 ± 1.2 A	8.5 ± 11.1 B	5.5 ± 11.1 B	344.5 ± 57.16 A	90 ± 0.00 A	90 ± 0.00 A

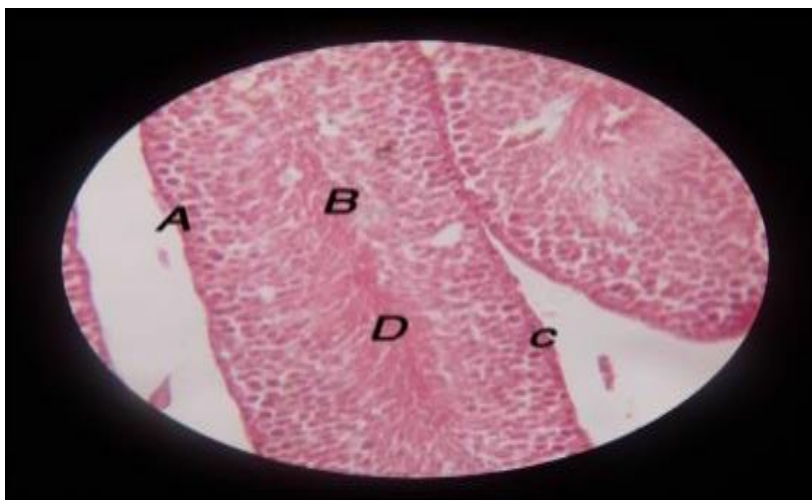
\*Different letters represent significant differences at ( $p \leq 0.05$ )

#### 4. Histological Changes

Injecting 5 mg/kg BW of G C seed oil extraction into male mice showed improvement in the testicular tissue, represented by narrowing of the testicular lumen by increasing spermatogenesis processes and, therefore, an increase in sperm numbers (Figure 2), as compared with the testicular tissue of control male mice Figure 1.



**Figure 1:** Histological Changes of Testes from The Control Group Show Normal Sperms (A) and Normal Connective Tissue (B. H&E, 40x)



**Figure 2:** Histological Changes in Testes from Male Mice Treated with GC Seed Extract 5mg/Kg B W Show Normal Connective Tissue (A), Increase in The Number of Sperms (B), Increase in Spermatogenesis (C), And Narrowing of The Testicular Lumen (D). H&E, 40x

## 5. Discussion

The results showed a significant increase in RBC count and Hb concentration when the GC seed oil extraction was administered to the animals. The blood parameter values were reduced significantly due to sodium nitrate injection. It seems that sodium nitrate increases the production of reactive oxygen species (ROS) (Halliwell *et al.*, 1997) that attack living cells and tissues, including the RBC and WBC (Sikka, 2004).

Increased generation of ROS causes lipid oxidation of the cell membrane of spermatozoa. Still, antioxidant vitamins play an essential role in protecting the cell from being damaged by ROS (Agarwal and Sekhon, 2010) and thus affect reproductive efficiency (Al-Aubody and Al-Diwan, 2014). The GC seed oil extraction contained flavonoids and phenols, considered potent antioxidants (Kasabe *et al.*, 2012). This explains the reason behind the improvements of the blood parameters values after significantly reduced by sodium nitrate due to the ability of the flavonoids to scavenge the ROS (Braun and Cohen, 2015) and may interfere with free radical formation. (Braun and Cohen, 2015) The improvements of the testicular tissue (figure 2) and the significant elevation in sperm count were due to the GC seed oil extraction containing the number of antioxidant vitamins A, E, and C (8, 17, and 1). It has been known that vitamin E improves semen viability and testicular tissue repair (Verma and Kanwar, 1999).

The combination of flavonoids and vitamin C increases both compounds' effectiveness (Mathiesen *et al.*, 1996). They delay the onset of lipid peroxidation (de Whalley *et al.*, 1990). Vitamin C is a major chain-breaking antioxidant that neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals, preventing sperm agglutination (Agarwal and Sekhon, 2010). In addition, it also helps recycle vitamin E (Sies, Stahl and Sundquist, 1992) and also has a role in stimulating spermatogenesis (Acharya *et al.*, 2008)

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