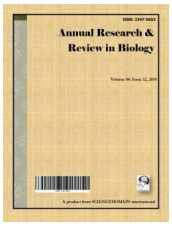

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Effects of *Eruca sativa* Leaves Extracts on Testes, Fertility Potential and Testosterone Concentration in Male Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: *Eruca sativa* (E.S.) is a worldwide herbaceous plant usually used for salad preparations and in traditional medicine for their therapeutic properties.
Aims: This study aimed to determine the effect of ethanolic extract of *Eruca sativa* leaves (E.S.) on testosterone levels, and histological changes of testes.
Methodology: Fourteen albino male rats were divided into two groups: Group A, rats served as controls. Group B, the rats dosed orally with 500 mg kg-1 of extract daily for 4 weeks. Student's t-test was used to compare the mean values of experimental group and control.
Results: The results showed that final body weight was significantly lower ($P<0.05$) than in the control. A significant increase ($P<0.05$) of testes weight was observed as compared with control. Significant differences ($P<0.05$) were also found in the mean concentration of testosterone between the experimental group and the control (6.294 ng/ml and 3.256 ng/ml, respectively).The dose caused a significant increase ($P<0.05$) of total protein and total cholesterol value, compared to the control. On the other hand, a significant reduction ($P< 0.05$) of triglycerides was observed. The histological study showed a decrease in the interstitial space, an increase in the diameter of

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seminiferous tubules, spermatid and leydig cells compared with the control.
Conclusion: Ethanolic extract of E.S. leaves could increase fertility potential and testosterone concentration in male rats. This plant seems to reduce serum cholesterol levels with some alterations in the structure and ultrastructure of the testes when this dose (500 mg kg^{-1}) has used.

Keywords: Eruca sativa; testes histology; albino rats; electron microscope.

1. INTRODUCTION

Eruca sativa (E.S.) most commonly known as rocket is a worldwide herbaceous plant usually used for salad preparations due to its astringent properties [1]. *Eruca sativa* belongs to the Brassicaceae family, is known in Jordan as Jarjeer. Previous studies showed that E.S has powerful active components that might be effective in human health and preventing cancer [2]. Rocket species are well-known in traditional medicine for their therapeutic properties as an emollient, diuretic, astringent, digestive, tonic, laxative, rubefacient, depurative and stimulant [2,3]. In addition, it has been extensively reported in the literature that E.S. has also antiephrolethiatic, antihyperlipidemic, antihyperglycemic and hepatoprotective activity [4]. Moreover, it is reported that rocket seeds contain vitamin C, carotenoids, flavonoids such as appiin, luteolin and glucosinolates the precursors of isothiocyanates and sulfaraphene [5] and volatile oils like phellandrene , apiole and myristicin [6,7]. Glucosinolates were found to have several biological activities including anticarcinogenic, antifungal, antibacterial plus its action as an antioxidant [8]. Many authors indicated that E.S. leaves possess a potent free radical scavenging antioxidants and protected against oxidative damage by increasing /maintaining the levels of antioxidant molecules and antioxidant enzymes [9,10].

Infertility is one of the major health problems in animals [11], approximately 30 % of infertilities are due to a male factor [12,13].Toxins, chemotherapy, drug treatment and environmental factors can have a harmful effect on spermatogenesis [11]. Medicinal plant for the treatment of diseases has a long tradition [14].

It was reported that E.S. might stimulate testicular steroid production, leading to increase spermatogenesis in the testis of male mice [15]. The ethanolic extract yields organosulfer compounds in which has anti carcinogenic, antiinflammatory and anti proliferative activity [16]. This extract possesses potential renal protective activity [9], support weight loss and helps regulate cholesterol levels [17,18]. The present study was performed to investigate the expected effects of ethanol extract of E.S. leaves on fertility hormone and on the histological structure and ultrastructure of the testis of male albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Design

This experiment was conducted at the Pathology Department of the Faculty of Medicine, the University of Jordan, Amman during 2015. Fourteen male albino rats were divided into two groups, each consisting of seven animals: the control-untreated group (group A) and the E.S. extract - treated group (group B). The E.S. treated rats were given orally 500 mg/kg body weight E.S extract daily until the end of the experiment (4 weeks). While the control rats group were given orally normal saline (volume equal to that of the dose of extract in the previous group) daily for 4 weeks.

Each group was housed and kept at 25℃ in 12h dark:12h light cycle and fed *ad libitum*. The study was approved by the Institutional Review Board of Al-Balqa Applied University. Performance and recommendations of animal procedures were applied according to the National Institutes of Health [19]. At the end of the experiment, rats were sacrificed, the testes were obtained and weighed. Blood samples were collected in clean, dry plain test tubes, and testes were immediately isolated from each animal and taken for histological and biochemical analysis.

2.2 Preparation of Plant Extracts

Eruca sativa plant were obtained from a local market in Amman. The leaves were air-dried, and then powdered. Extraction by refluxing with 100 ml analytical grade of ethanol solvent (Sigma, Germany) for each 30 g powdered plant material was performed for two weeks. After that, filtration of extracts was done using filter paper and White canvas. Thereafter, solvents were

evaporated to dryness. The next step was weighing the crude extracts before dissolving it in 0.05% dimethyl sulphoxide (DMSO) (Sigma, Germany) to a final stock concentration of 1g/ml. Finally, filtration of the extracts using 0.22 µm filter units to purify it before keeping it at -20℃ until use.

2.3 Light Microscopic Study

The procedure used in light and electron microscopy is described in full earlier in the literature [20]. Samples of each testis were fixed in 10% formaldehyde for 1 day, processed, embedded using paraffin wax then sectioned by rotary microtome (Leica RM 2125 RT, Germany). Finally, the 5µm sections were stained with Hematoxylin & Eosin (Hx & E) and examined under a compound light microscope.

2.4 Electron Microscopy

Specimens of $1mm^3$ were taken from testes. Tissue specimens were then fixed in glutaraldehyde (3%) (Sigma, Germany) in 0.1 M sodium phosphate buffer, pH 7.3, for 24 hours at 4Cº and osmicated in osmium tetroxide (2%) (Sigma, Germany). After that, dehydrated by graded acetone series and embedded in Araldite medium and left in for 1 day and finally sectioned by ultramicrotome (Leica EM UC7, Germany) to get ultrathin sections. As a preliminary step, stained semithin sections (with toluidine blue stain) were examined under compound light microscope. Ultrathin sections were put on grids and stained with lead citrate and uranyl acetate then were examined using transmission electron microscope (TEM) (Morgagni 268, Holland).

2.5 Biochemical Study

Blood samples were collected directly from the eye in plain test tubes, centrifuged at 2500 rpm for 5 minutes. Then the obtained serum was stored at - 80℃ until use. Measurements of serum level of testosterone were determined using a free testosterone enzyme immunoassay test kit (Dia Metra, Foligno, Italy).

2.6 Statistical Analysis

Student's t-test was used to compare the mean values of experimental group and control. All values were presented as mean ± SD. Differences were considered significant at $P < 0.05$.

3. RESULTS

At the beginning of the experiment body weight gradually increased in the treated group as compared with control. As shown in Table 1, final body weight was significantly lower ($P < 0.05$) than in the control. A significant increase ($P< 0.05$) of testes weight was observed as compared with control. Significant differences ($P < 0.05$) were also found in the mean concentration of testosterone between the experimental group and the control. The dose caused a significant increase ($P< 0.05$) of total protein and total cholesterol value, compared to the control. On the other hand, a significant reduction ($P< 0.05$) of triglycerides was observed.

Table 1. Effects of *Eruca sativa* on body weight , testes weight and biochemical parameters of rats after 4 weeks

Parameters	Group A (control)	Group B (*Eruca Sativa* Leaves Extract) dose 500 mg kg^{-1} BW
Body weight		
initial weight	191.0 ±13.56 [a]	195.0± 8.94 [a]
final weight	259.2±7.87 [a]	241.4 ±27.20 [b]
difference (g)	68.2	46.4
difference %	35.71	23.79
Testes weight		
Testes weight (g)	2.760 ± 0.47 [a]	2.980 ±0.24 [b]
Testosterone concentration (ng\ml)	3.256 ± 2.86 [a]	6.294 ± 3.71 [b]
Biochemical parameters		
Total Protein	5.862 ± 1.32 [a]	7.778± 0.30 [b]
Total Cholesterol	76.200 ± 9.91 [a]	80.200 ± 14.99 [b]
Triglycerides (TG)	82.200 ± 20.38 [a]	62.200 ± 7.47 [b]

1: All readings are means ± Standard Deviation of seven determinations.
2: Values with different letters in the same row are significantly different from each other (P < 0.05).

Histological examination of cross sections of testis in control group stained with hematoxylin and eosin (Fig. 1 a.) revealed that normal architecture seminiferous tubules epithelium showing all stages of spermatogenesis. While in testis sections of treated group enlarged seminiferous tubules were observed (Fig. 1 b.) and their lumen were filled with sperms (Fig. 1 c.). Not only increase in the diameter of seminiferous tubules, the abundance of seminiferous tubules with proliferated, differentiated and matured spermatozoa but also the walls of some tubules were found vacuolated as well as interrestial space with some abnormal leydig cell and some elongated spermatid (Fig. 1 d.) as compared with the control group. These results were confirmed by sections obtained and studied using transmission electron microscopy.

Fig. 2 showed electron micrographs of the testes of control and treated rats. Normal epithelial cells that constitute the seminiferous tubules of a control group with normal spermatid and normal spermatozoa are seen (Fig. 2a). The abnormal ultrastructure in seminiferous tubules of some treated rats testicular tissue was recorded. The obvious histological changes were abnormal acrosome of spermatid (Fig. 2b.), abnormal interistial cells (Fig. 2 c.) and extended to affect mitochondria since it was founded as degenerative mitochondria (Fig. 2b. & c.). Adding to these changes some elongated sperms in

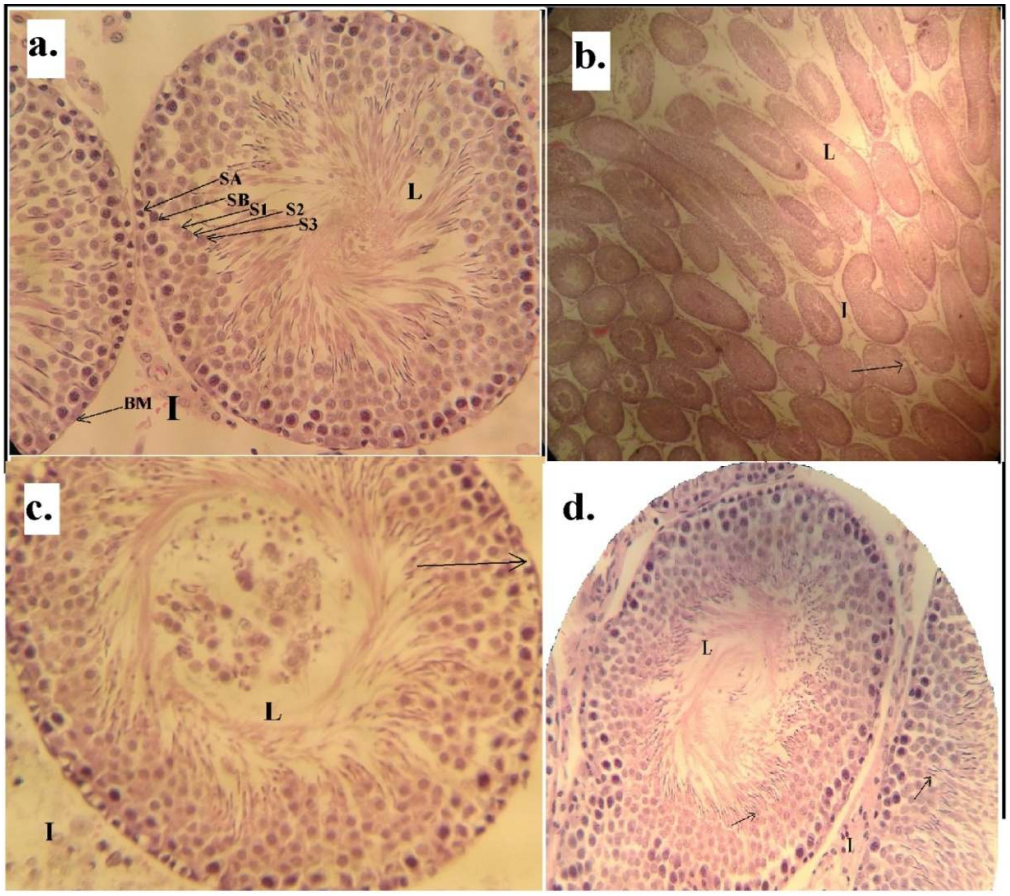

Fig. 1(a-d). Light photomicrograph of the testes of control and treated rats.

(a) Normal structure of control testicular tissue with active spermatogenesis in seminiferous tubules. L: Lumen filled with matured spermatozoa. BM: basement membrane. SA: spermatogonium type A. SB: spermatogonium type B. S1,S2and S3 : all stages of spermatogenesis. (H&E stain, 400x) , (b) testicular tissue from treated group showing seminiferous tubules variation in size and shape. L: Lumen . I: interrestial space with ledyg cell.→: vacuole in the wall of the seminiferous tubule. (H&E stain, 100x) ,(c) testicular tissue from treated group showing seminiferous tubules with plenty of spermatozoa in its lumen. L: Lumen filled with spermatozoa.I : interrestial space with abnormal ledyg cell. →: vacuole in the wall of the seminiferous tubule. (H&E stain, 400x) , (d) testicular tissue from treated group showing seminiferous tubules with plenty of spermatozoa in its lumen and elongated spermatid. L: Lumen filled with spermatozoa. I : interrestial space with abnormal ledyg cell. →: elongated spermatid. (H&E stain, 400x)

lumen of seminiferous tubule were reported (Fig. 2 d.).

4. DISCUSSION

This research demonstrated that administration of ethanolic extract of E.S. leaves at the dose of 500 mg/kg for 4 weeks, caused a significant increase in fertility parameters, significantly decreased final body weight of rats, and the weight of testes increased significantly ($P<0.05$) when compared with that of the control group. These results are in agreement with those reported by Farman [21], who found that the treatment of male albino mice with the extract of E.S. led to a significant increase in the weight of testis. Shams Al-dain and Jarjeis [22] found that supplementation with 600 mg E.S. seeds/kg BW/day in Awassi lambs rations had improved the studied hematological and biochemical parameters. The obtained results are in accordance with those reported by Hussein [23] who found an increase in testosterone level in mice treated daily with 30 mg/Kg BW and 40 mg/Kg BW of E.S. leaves aqueous extract for five weeks.

The present histological study revealed that the administration of 500 mg/kg BW of the E.S. leaves caused few alterations in the seminiferous tubules when administered for 4 weeks. The examined testicular sections showed alterations from normal histology such as increase in the diameter of seminiferous tubules, spermatid and leydig cells. These alterations might be a result of stimulatory effect of this plant in the growth and proliferation processes in testis as well as its ability to increase the activity of spermatogenic cells [24]. These results are also in agreement with those reported by Salem and Moustafa [25]

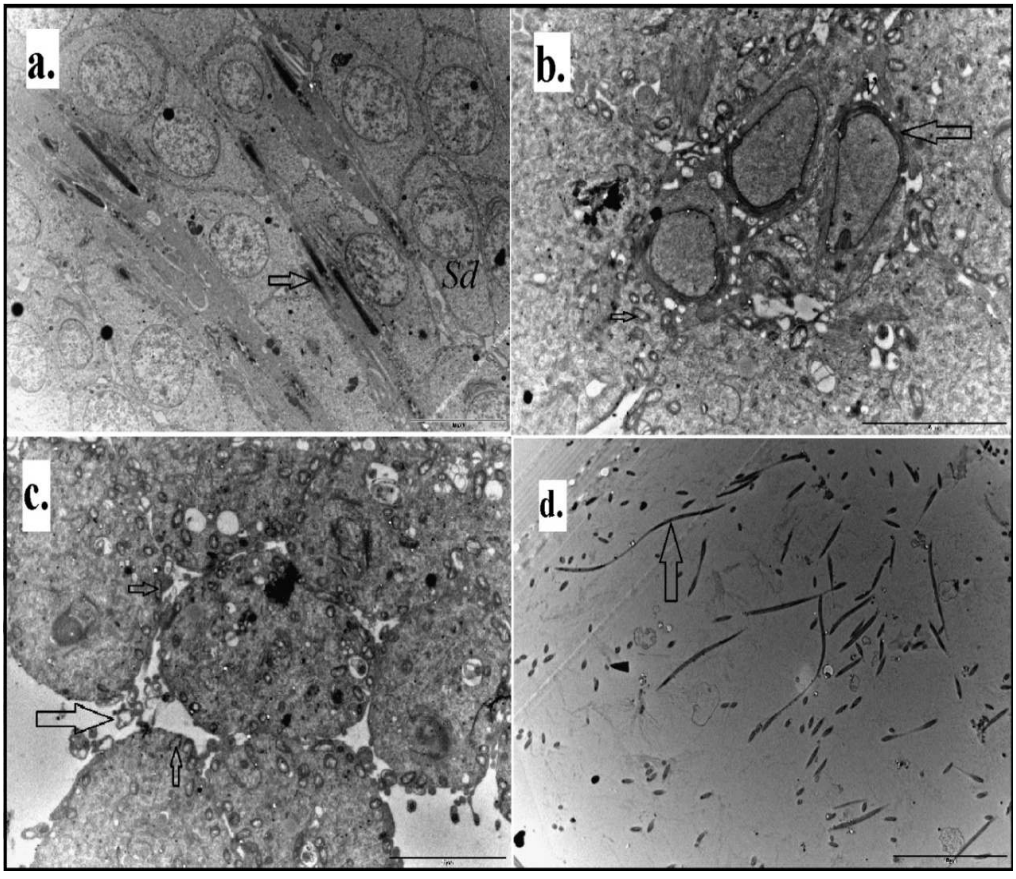

Fig. 2(a-d). Electron micrographs of the testes of control and treated rats.

(a) Normal ultrastructure of seminiferous epithelium from control rats, normal spermatid (sd) and normal spermatozoa (arrow). (1800x), (b) abnormal acrosome of spermatid(large arrow)in testis treated rats, abnormal and degenerative mitochondria (small arrow). Vacuoles (v). (5600x), (c) degenerative mitochondria (small arrow) in seminiferous tubules of treated rats. abnormal interistial cells (large arrow). (4400x), (d) elongated sperm in lumen of seminiferous tubule (arrow) of treated rats (2200x)

who used low dose (0.25 ml/Kg) of E.S. seed oil 3 times per week for 6 weeks. Moreover, the results of Ravet et al. [26] supported this observation.

Many plants have been studied for the possible fertility regulatory properties [27]. Some medicinal plants are used as a fertility enhancing agents [28,29,30]. It is reported that E.S. seed oil is widely used by males to improve their sexual performance, the valuable effects of the oil are usually related to its fatty acid contents such as erucic acid [25]. Hussein [23] and Salem and Moustafa [25] demonstrated that E.S. leaves which contains steroids, alkaloids, flavonoids, terpenes, glycosides and saponins and seed oil has beneficial effects on fertility and male reproductive system. Moreover, several studies have shown that the aqueous extract as well as ethanolic extract of E.S. plant increase spermatogenesis [23,31]. Intake E.S. leaves as a salad may be helpful for diabetic patients to minimize the reproductive deterioration [32]. Limitations of the study included the small number of rats and the short duration of the study . It is recommended that further studies aimed at corroborating these findings be carried out.

5. CONCLUSION

The present study showed that daily oral administration of 500 mg/kg body weight ethanolic extract of E.S. leaves for 4 weeks could increase fertility potential and testosterone concentration in male rats. This plant seems to reduce serum cholesterol levels with some alterations in the structure and ultrastructure of the testes when this dose is used.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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