

Study the Effect of *Eruca Sativa* Leaves Extract on Male Fertility in Albino Mice

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Abstract

This study was designed to determine the effect of *Eruca sativa* leaves extract on testosterone levels, sperm activity, mortality and abnormalities, and histological changes of testes. The aqueous leaves extract of *E. sativa* was prepared and then chemical detection was done. Results revealed that alkaloids, glycosides, flavonoids, saponins, coumarines, resin, terpenes and steroids were present in *E. sativa* extract. The fertility effect was carried out by treating mice with two doses (30 and 40) mg/Kg of extract. Results showed a significant ($p \leq 0.05$) increase in testosterone level, sperm activity and a significant decrease in sperm mortality and abnormalities were recorded. Histological changes in testicular section exhibited a significant increase in the diameter of seminiferous tubules, spermatid and Leydig cells. A significant decrease in the interstitial space was noticed.

Keywords: *E. sativa* aqueous extract, male fertility, histology.

Introduction

All civilizations have always had traditions of using herbs to promote healing. Plants still remain the basis for development of modern drugs and medical plants have been used for years in daily life to treat diseases all over the world [1]. *E. sativa*, also known as arugula, or rocket, is called "Jarjeer" in Arabic, is an edible plant; Rocket is also considered a medical plant with many reported properties, including its strong aphrodisiac effect known since Roman times [2, 3].

Rocket used medicinally, in early times, Jabir described its use in a plaster, to draw out poison, including scorpion venom, while al-Kindi includes rocket seed in a stomach ache and in a remedy for insanity [4]. It is used in oil form as a treatment for hair loss and as an ointment, it is used for treatment of burns [5]. Oil of *E. sativa* seeds is tried for prevention and treatment of diabetes mellitus induced experimentally by alloxan injection in rats [6] and possess a potent antioxidant and renal protective activity and preclude oxidative damage inflicted to the kidney [7]. Recent studies suggest that Rocket have already shown anticancer activities [8].

Materials and Methods

Plant was bought from a local market. The leaves were air-dried, and then powdered using a coffee grinder. Fifty grams of the leaf

powder were extracted for three hours in 250 ml of the distilled water using the Soxhlet apparatus and the source of heating was a warm water bath (45°C). The leaf extract solution was then evaporated at 45°C using a rotary evaporator, and the resultant crude extract was frozen at -20°C until use to prepare the required doses [9].

Detection of some active compounds in *E. sativa* leaves extract

Detection of active constituents in rocket leaf extracts was carried out according to alkaloids and glycosides [10], flavonoids and resins [11], saponins [12], Coumarines, terpenes and steroids [13].

Experimental Design

Twenty four albino male mice were divided into four groups designated as A, B and C. Each group consisted of 8 mice, and subjected to the following treatments:

Group A : Mice treated with 0.1 ml of physiological saline.

Group B : Mice treated with 0.1 ml extract at dose of 30 mg /kg body weight.

Group C : Mice treated with 0.1 ml extract at dose of 40 mg/kg body weight. Mice dosed with 0.1 ml of extract daily by oral administration for 5 weeks.

Testosterone estimation

Blood was collected by heart puncture by putting the mouse under anesthetic conditions and the needle was at acute angle to avoid rupture of RBCs. Serum was separated by centrifuging at 2000 g for 10 min., and then was stored at 4°C until use. Mice were sacrificed by cervical dislocation and tests from each group was isolated. Serum level of testosterone was measured as the procedure described by manufacturer according to the method mentioned by.

Collection and preparation of sperms

Mice were killed by cervical dislocation and dissected directly, the testes were removed and placed in a sterile disposable Petri dish containing 0.9 ml RPMI-1640 medium, the sperms were collected from the epididymis of mice by the caudal was cut and placed in a petridish containing 0.9 ml of RPMI-1640 medium and minced by using microsurgical scissor and forces [14] [15].

Sperm motility

Sperm motility was assessed according to the method reported by [15] Fifty µl of the sperm suspension was placed over a slide and covered by a cover slide. Using light microscope, several fields were examined to estimate the percentage of individual motility of sperms [16].

Sperm mortality and abnormalities

The percentages of dead and abnormal sperms were measured as following according to the method reported by [16]. A drop of the sperm suspension was placed over the slide and, then a drop of eosin-nigrosin stain was added and mixed. The mixture was spread using another slide and left to dry. Using light microscope, 200 sperms were counted to calculate the percentages of dead and abnormal sperms as in the following equation:

$$\text{Percentage of Dead sperms} = \frac{\text{NO. of dead sperm}}{\text{Total sperm}} \times 100$$

Histopathological studies

Pieces taken from testes were placed in Petri dishes containing physiological salt solution to remove the fatty tissues and sticky bundles, then the organs were put in tubes containing 10 % formalin for about 16-18 hrs for fixation purpose, then they were transferred into tubes containing 70% ethanol

alcohol in which they were preserved till the time of the final preparation [15]. The staining method was performed by using hematoxylin and eosin[16].

Statistical analysis

The values of the investigated parameters were analysis of variance (ANOVA) and Duncan test, using the computer programme SPSS. Version (7.5) given in terms of mean ± standard error, and differences between means were assessed [17].

Results and Discussion

The aqueous extract of *E. sativa* was subjected to chemical analysis and results indicated that alkaloids, terpenes, flavonoids, saponins, glycosides and steroids were present in the extract (Table (1)). Similar results were obtained by [18].

Table (1)
Chemical detection of some active compounds in ErucasativaLeaves extract.

<i>Type of secondary metabolite</i>	<i>Result of detection (Aqueous extract)</i>
Alkaloid	+
Glycosides	+
Flavonoids	+
Saponins	+
Tannins	-
Coumarines	-
Terpens and steroids	+
Resins	-

+ve indicates the presence of secondary metabolites.

-ve indicates the absence of secondary metabolites.

Results exhibited that rocket leaves extract caused a significant increase in testosterone level, sperm activity; moreover a significant decrease in sperm mortality and abnormalities was recorder. This result agreed with [18] who reported that the presence of saponine, alkaloids in rocket extract caused a significant increase in sperm activity. Results indicated that there were no significant differences between group B and C Table (3).

Table (2)

Testosterone level abnormalities in mice treated with two doses of Erucasativs leaves extract and control.

Group (n=8)*	Testosterone ngm/ml Mean ± SE *
Group A	0.46± 0.06 a
Group B	0.60± 0.03 b
Group C	0.62±0.05 b

*Different letters means there is a significant difference ($p \leq 0.05$) as compared with control.

* n= number of animals per group used during the two weeks of the experiment.

Table (3)

Sperm activity, mortality and abnormalities in mice treated with two doses of Erucasativs leaves extract and control.

Group (n=8)	Sperm activity Mean±SE*	Sperm mortality Mean±SE	Sperm abnormalities Mean±SE
Group A	69±4.18 a	28± 4.03 a	23 ± 3.97 a
Group B	72± 2.73 b	20 ± 2.58 b	18± 3.49 b
Group C	78.9±5.70b	20 ± 2.55 b	17 ± 1.48 b

*Different letters means there is a significant difference ($p \leq 0.05$) as compared with control.

* n= number of animals per group used during the two weeks of the experiment.

Histological sections of seminiferous tubules after five weeks of treatment with *E.sativa* extract caused a significant increase in the diameter of seminiferous tubules, spermatids and leyding cells, and decreased the interstitial space was noticed When compared to the control group (Table (4) and Fig.(1)).

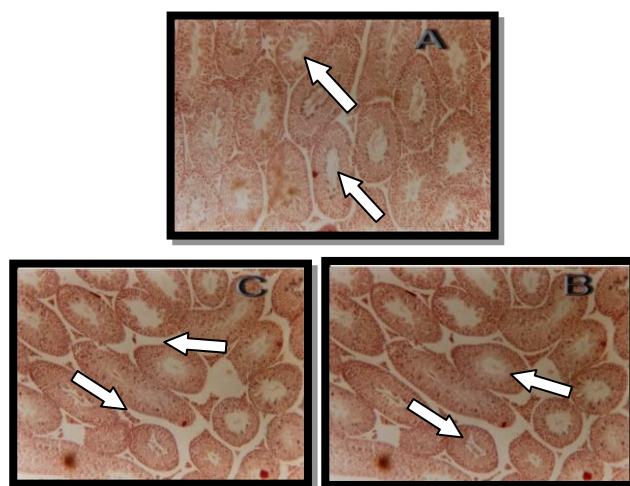


Fig.(1) Section of testes showing: (A) seminiferous tubules of normal mice (B) seminiferous tubules of mice treated with 30mg/kg of extract showed a large diameter (C) seminiferous tubules of mice treated with 40mg/kg of extract showed a large diameter and decrease the interstitial space (400X)(arrows).

This increase might be due to ability of *E. sativa* extract to stimulate the growth of testes and enhance the proliferation, maturation and differentiation of spermatozoa as compared with the control group [19].

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الخلاصة

اجريت الدراسة الحاليه لمعرفة تاثير المستخلص المائي لاوراق الجرجير على مستوى هرمون الشحمون الخصوي وفعالية النطف والنطف الميته والمتشوهه والتغيرات النسيجه على الخصى وحضر المستخلص المائي لنبات الجرجير واجري الكشف الكيمياءى للتعرف على مجاميع المركبات الفعاله وهي مركبات القلويدات والكلايكوسيدات والفلافونات والصابونيات و الكورماينات والراتنجات والتربينات والسترويدات والتاثير على الخصويه تم التعرف عليه بمعاملة الفئران بتركيزين (٣٠ و ٤٠) ملغم / كغم اوضحت النتائج زياده معنويه في هرمون الشحمون الخصوي وفعالية الحيامن وانخفاض معنوي في النطف الميته والمتشوهه والتغيرات النسيجه في مقاطع الخصى سجلت زياده معنويه في اقطار النبيبات المنويه والارومات النطفيه وخلايا لايدك ولوحظت انخفاض معنوي في المسافات البينييه بين النبيبات المنويه