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Effect of alcohol extract of watercress (*Eruca Sativa*) on human sperm motility during*in vitro* sperm activation

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Abstract
Background:
Herbal plants often contain active pharmacological compounds they are commonly used for the treatment and prevention of different diseases.
Objectives
The present study was aimed to identify the effects of different concentrationsof Erucasativa extraction (ESE) on human sperm motility for infertile male in vitro.
Methods: *Thirty five semen samples were obtained from men (mean age 27, range from 20-35 years) and prepared using centrifugation swim-up technique. Pro-SMARTmedium was used only in group1 (G1) as controls and two concentrations of ESE (50µg/ml and 100 µg/ml) were add to the culture medium of groups 2(G2) and3(G3) for in vitro sperm activation*
Results: *Present study revealed that the sperm motility (%)was significantlyincreased($P<0.05$)post activation as compared to pre activation. Generally, group G3 showed best percentage of progressive sperm motility than G1 and G2.*
Conclusion
The high concentration of ESE (100µg/1mL) improved humansperm progressive motility during sperm activation in vitro.
Key words*: Eruca sativa, human sperm,centrifugation swim-up technique, pro-SMART.*

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I. Introduction

Many studies around the world were performed on herbal plantsfor possible regulatory properties of fertility [(1)]. Commonly herbal plants are used to relieve sexual dysfunction as aphrodisiac, or as agents improving fertility. They enhance sexual performance suchas via providing of nutritive value[(2, 3)].

Actually,*E. sativa* is an eatable as vegetable or spice, also called rocket, in Arabicwhich isnamed "Jarjeer". Rocket with many reported properties is considered a medical plant such as antimicrobial, renal protective activity, antihyperlipidemic, strong aphrodisiac effect [(4, 5)]. Furthermore, ancientArab, used*E sativa* seed in a stomach ache and in a therapy for psychosis, while others described it's to draw out poison, use in a plaster, such as scorpion poison [(6)]. Although diabetes mellitus experimentally induced by alloxan injection in rats is tried Oil of *E sativa* seeds for prevention and treatment [(7)], while in hair loss and burns it is used ointment for treatment [(8)].

Leaves and seeds of*Eruca sativa* thatpossess a potent antioxidant and prevent oxidative damageby increasingthe levels of antioxidant enzyme[(9)].Other studies that showed that Rocket have anticancer activities[(10)]. Therefore, the present study was aimed to identify the effects of different concentrations of *Eruca sativa* extraction (ESE) on human sperm motility for infertile male *in vitro*.

II. Materials and Methods

1-Preparation of alcoholic *Eruca sativa* leaves extract:

Freshvegetable leavesof *Eruca sativa* were bought from a local market; the leaves were dried in the shaded place for 7-10 days and then powdered using electrical blender. 50g amount ofrocket leaves were placed in a glass percolator with 500 ml of ethanol and were allowed to stand at room temperature for about 72 h. After 3 days the mixture was filtered by usingWhatman filter paperandfiltrate extract was concentrated by rotaryevaporator [(11)].

2-Semen sample collection

This study was carried out in the laboratories of the Higher Institute of Infertility Diagnosis and assisted Reproductive Technologies at AL-Nahrain University during the period from January to April, 2017. Thirty five

semen samples were obtained from male withmean age 27, and range from 20-35 yearsand collected by masturbation in a dry, clean, and sterile disposable Petri-dish after 3-5days period of abstinence in quite private room adjacent to thelaboratory of semen analysis. The container was labeled with the following information, name, age, abstinence period and time of sample collection. The samples were placed at 37Cfor 30 minutes to allow liquefaction in an incubator [12, 13]. The liquefied semen was carefully mixed for few seconds, and then the specimen was examined in detail by macroscopic and microscopic examination within one hour of collection [14].

3-Technique of *in vitro* human sperm activation

Centrifugation swim-up technique was applied in this study. After liquefaction each semen sample was prepared for SFA, then divided into three groups, as control group(G1), low dose ESE (G2)and high dose ESE (G3), these 3 groups of semen samples washed with culture medium and centrifuged for 6- 7 minutes at 2400 R.P.M. The upper layer was discarded. Add 1mL pro-SMART medium in sperm pellet slowly in control group (G1). 1mL of the prepared pro-SMART medium supplied with one of two respectively doses of*Eruca sativa* extraction (50µ g/1mLand100µg/1mL) wereadded to the sperm pellet in treated groups G2 and G3 groups.
After incubation for 30 minute at 37C, one drop from the upper layer was aspirated by Pasteur pipette. Then, examined under light microscope at (400 x) magnification for assessment of sperm parameters.

5- **Statistical analysis.**
Means and standard error of mean (mean +SEM)were determined by using statistical descriptive method. MANOVA test was used to compare among different means. The data were statistically analyzed by SPSS version 24[15].

III. Results

Table (1) show the percentages sperm motilityfor(G1,G2and G3) post activation which appeared significantincreased (P<0.05) as compared to pre-activation.Whereasthey shownon significantdifferences (P>0.05) between G2and G3. In general, sperm motility(%) for G1 group was significantly reduced (P<0.05) as compared to G2and G 3groups.
Table (1) in vitro sperm activation using centrifugation swim-up technique and media enriched with tow concentration of eruca sativa extraction (100µg/ML, 50µg/ML)

Sperm parameters	Pre- activation group	Post-activation		
		Control group-G2	Low ESEdose-G3	HightESEdose-G4
Sperm Motility (%)	40.333 + 2.00	79.555 a + 2.18	87.555 a;b + 1.67	88.777 a;b + 1.58
Progressive sperm motility (%)	18.111 + 1.08	39.888 a + 2.19	47.444 a;b + 1.80	50.740 a;b;c + 2.04
Non-progressive sperm motility (%)	22.222 + 1.312	39.666 a + 1.89	39.963 a + 1.33	37.851 a;c + 1.56
Immotile Sperm (%)	59.666 + 2.00	22.185 a + 2.82	13.629 a;b + 2.55	13.222 a;b + 2.73

a: means significantly different as compared to pre-activation G1 group.
b: means significantly different as compared to G2 group.
c: means significantly different between G3 and G4 groups.
*: Similar letters means non-significant differences

A significantincrease (P<0.05) was reported inthe percentages of progressive spermmotility (%) post activation groups in relating G1, G2and G3as compared with pre-activation group. Also, they showed significant differences (P<0.05) among all groups of post activation. In general, progressive sperm motility (%) for G3 group was the highest as compared to G1and G2 groups.While, G1roup was significantly reduced (P<0.05) as compared to G2 group

Non progressivespermmotility (%) showed a significant increased (P < 0.05) in G1, G2and G3 post activation) as compared to pre-activation after using centrifugation swim-up activation technique.Whereasnon significantdifferences (P > 0.05) were seen between G1 and G2.On other hand, a significant decreased (P < 0.05) between G3and G2 groups as compared to G3group.

Thepercentageof immotile sperm revealed a significantdecreased(p < 0.05) for all post activation groups as compared to pre-activation group.whereasnon significant differences(P > 0.05) were seen between G2 and G3 groups. In contrast significantincrease in G1group as compared to G2, G3groups.

IV. Discussion

Sperm activation is very essential step in assisted reproductive technologies, that animportant to determining the outcome on it [(16)]. in the present study, the sperm function was improved in human sperm motility and progressive sperm motility, that related to the culture media and method for sperm preparation can enhancedsperm function in assisted reproductive technologies [(17)]. In addition to reported that only the activation motile sperm will swim-up to the superior area during activation using centrifugationswim-up technique [(18)].

In present studyreduction in immotile sperm(%) wasshowedfor all semen samples were examined post-activation as compared to pre- activation. However,this result may be belong to the preparation method, immotile spermatozoa and semen debris stay in pellet meanwhile, the good quality spermatozoa were picked up from upper layer and after activation were absent in bad quality spermatozoa[(18)]and this result was agreed with Hilo[(19)].

In the current study, the percentage of progressive sperm motility is directly related to treated with ESE, this may be due to the chemical composition such assaponins,terpenes, flavonoids, steroids,alkaloids and glycosides were present in the extract were obtained by [(20)]. Moreover, the presence of some trace elements (Cr,Cu,Fe,Mn and Zn) in the leaves of this plant [(21)].Copper (Cu) has been shown to be important for the activity of an enzyme responsible for removing toxic free radicals. (Cu-Zn superoxide desmotase) as well as for the activity of phagocytes [(22)]. Furthermore, Jarjeer is act as a good antioxidants source, such it is contains glucosinolates, carotenoids, phenolic compounds, and degradation products, as isothiocyanates[(23)]. Therefore, from the results of this study revealed that the sperm motility (%) significant increased post activation as compared to pre activation. Generally, group G3 showed progressive sperm motility best thanG1 and G2.

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