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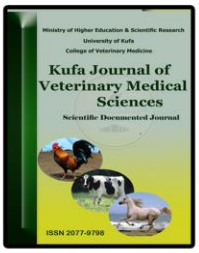

Study The Effect of Two Crude Ethanolic extracts of *Eruca sativa* and *Zingiber officinale* in comparison with Tadalafil on Sperm Parameters in Rats

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Abstract

Two ethanolic extracts of *Eruca sativa*(Rocket) and *Zingiber officinale*(Ginger) were studied after 42 days of treatment with effective dose (ED)of 200 mg per kg of BW for rocket and 400 mg per kg of BW for ginger. The results indicate significant increase in sperm count for groups of rocket and tadalafil, while significant decrease in sperm motility for tadalafil as compare with control, however, no indication of significant change in sperm abnormalities for all groups as compare to control.

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 [Ates, and Erdogrul, 2003]. Rocket is also considered a medical plant with many reported properties, including its strong aphrodisiac effect known since Roman times [Padulosi, and Pignone, 1997; Font et al., 2003]. Barillari et al., (2005)who reported that the presence of saponine, alkaloids in rocket extract caused a significant increase in sperm activity. However, also, the aphrodisiac characteristics of rocket have been attested since ancient Egyptian and Roman times (Fernald, 1993; Michael et al., 2011). In Arabian medicine, ginger is considered an aphrodisiac (Qureshi et al ,1989). Also, Khaki et al., (2009) reported that administration of ginger significantly increased sperm percentage, viability, motility and serum total testosterones in rats.

Materials and Methods:

Extract preparation:

Ginger was obtained from commercial source from Najaf city ,and voucher specimen of the plant to be identified and authenticated at the National Herbarium of Iraq Botany directorate in Abu Graib-Baghdad , under certificate name *Zingiber Officinale* belongs to family Zingiberaceae, the root powder of ZO was put in thimbles that used in multi-units of soxhlet apparatus to method described by Harborne (1984), with 70% ethanol for 24 hours(Complete circulation) .The combined ethanolic extract was filtered , and the filterate was concentrated by a rotary evaporator under a

temperature not exceeding 40 C and a speed 80 rpm .

The leaves of Eruca Sativa was purchase from local planter ,that cultived in Najaf city .The plant classification was done at the Ministry of Agriculture of Iraq/State board of seeds testing and certificate, the powder of ES was prepared by the same procedure which followed with ZO.

Administration of ZOE, ESE and tadalafil:

After 42 days of treatment with ZOE, ESE, and tadalafil , that dissolved in DMSO as vehicle , control group was treated with DMSO only.

Preparation of Sperms

After the end of experimental three, animals were weighted by sensitive balance , then animals were anesthetized by intramuscular injection of (Ketamine HCL 90 mg per kg B.W. & Xylazine 40 mg per kg B.W.).

After cervical dislocation of animals, the tail of the left epididymus were taken and embedded in one ml of normal saline at $37C^0$ in a glass watch, and then the tail was cut into at least 200 sections by microsurgical scissor, to perform the following microscopically examination on sperm characters.

Microscopic examination:

1:Sperm motility:

Sperm motility was measured according to the method reported by Bearden and Faquay (1992), by taking 50µl of the sperm suspension which was placed over a slide and covered by a cover slide.

By using light microscope , several fields were examined to estimate and count the percentage of individual motility of sperms ,that assessed according to the method reported by Ijam et al.(1990).

2:Sperm concentration (Sperm count):

Sperm count was done according to Sakamoto and Hashimoto (1986).By using Hemocytometer (Neubaur Type).The Hemocytometer slide were filled with 5µl of a sperm suspension and covered by cover slide ; the sperms were counted in twenty five small squares of the chamber .

Estimation the sperm was made according to the following formula:

Sperm concentration =Number of sperm × 100

3:Abnormal sperm morphology:

The method of Siegmud, (1979) was used to evaluate abnormality sperm morphology, by taking a drop of sperm suspension prepared and was placed over the edge of slide and then a drop of Eosin-Nigrosin stain was added and mixed , then two smears of each sample were prepared on microscopic slide . Viable sperms repel the vital stain (Eosin-nigrosin), Where as dead sperms had lost the structural integrity of their plasma membrane and therefore absorbed the dye(Smikle and Kuttan,1997).

The smears of sperms were examined for abnormal morphology, 200 sperm were counted in each smear, the final percentage was calculated by taking the average of two smears.

Abnormal sperm morphology was calculated as in the following equation:

Percentage of morphologically abnormal sperms=

(No. of Morphological abnormal sperms/Total sperms No.) × 100

The morphologically abnormal sperms were estimated by depending on sperms abnormality which had tailless head, tapered head, coiled tail, bifurcate tail, broken tail, and micro-head.

Statisyical analysis:

All data were expressed as mean ± SE . Student's t-test was

performed by Statistical Package for the Social Science (SPSS Inc. Chicago, IL,USA) software (version 15.0) with level of significance set at $P<0.05$.

Results:

The results of sperm parameters for roots of *Zingiber officinale* extract (ZOE) and leaves of *Eruca Sativa* extract (ESE) with Tadalafil were as following(Table 1):

Table1:Sperms evaluation parameters in rats.

Treatments	Mean ± SE		
	Sperm count (10^4/ml)	Sperm motility (%)	Sperm abnormality (%)
Control	64.0 ± 3.61 B	35.0 ± 2.09 A	35.0 ± 1.79 A
ZOE	58.0 ± 3.21 B	32.0 ± 1.75 A	34.0 ± 2.00 A
ESE	76.0 ± 4.07 A	35.0 ± 2.66 A	34.0 ± 2.11 A
Tadalafil	74.0± 3.92 A	20.0± 1.48 B	34.0±2.05 A
LSD value	7.882 *	8.329 *	5.063 NS
* (P<0.05) , NS: Non-significant.			

Discussion:

There was no significant differences in all groups of treated rats with control group in abnormality of sperms, but for sperm count ,there was significant increase in tadalafil and ESE groups as compare to control and ZOE groups, for ESE ,this corroborate the research of Salem and Moustafa, (2001) and Hussein, (2013) who concluded that *Eruca sativa* may be capable in improving healthy sperm parameters and fertility. For tadalafil, this result corroborate with study in guide of European Medicines Agency that a decrease was observed in sperm count and concentration related to tadalafil treatment of unlikely clinical relevance. In addition, for sperm motility significant decrease level of percentage of tadalafil comparison to other three treated groups, but this may disagree with Sikka and Hellstrom, (1991) who stated the Phosphdiestrase 5 Inhibitor(PDE5I) have good positive effect on sperm motility , may between the intracellular levels of cGMP and sperm ability to move, also this idea is in agreement with Pomara et. al.,(2007) that revealed a significant decrease in sperm motility after a single dose of tadalafil. The authors have suggested that the stimulatory effect on sperm motility may be due to a direct action of active ingredients on sperm mitochondria and calcium channels, which is in agreement with Cuadra et. al., (2000) that suggest a stimulatory effect on sperm motility , when PDE5I is moderately inhibited; however it exhibits extensive inhibition to sperm motility after long treatment.

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