Using of ELISA Technique for Detection the Supplement Effects of *Eruca sativa* Leaves in Human Males Hormones

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Abstract

This study was carried out to investigated the effect of *Eruca sativa* fresh on fertility potential,testosterone and progesterone hormone in human male. Sixteen male human were randomly divided into 4 group, control, group A, group B, and group C. each group comprising of 4 male divided into four age period. At the end of treatment period blood testosterone and progesterone concentration were in ensured by ELISA methods. Significant difference in blood testosterone concentration was observed in male group compared to the control group. The result of this study showed that *Eruca sativa* fresh leaves especially in higher doses could increase testosterone concentration in male human.

Keywords: ELISA Technique, *Eruca sativa*, Human Males Hormones.

استعمال تقانة الاليزا في الكشف عن تاثير تناول اوراق الجرجير في مستوى الهرمونات الذكرية

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الخلاصة:

قد أجريت هذه الدراسة للتحقق من تأثير اوراق نبات الجرجير الطازج على الخصوبة. وتاثيره على هرمون التستيرون و البروجسترون في الجنس الذكري (الانسان).تم اختيار ستة عشر من الذكور وتم تقسيمهم بشكل عشوائي إلى4 مجاميع، مجموعه السيطرة، مجموعة A، مجموعةB ، ومجموعة C. كل مجموعة تتألف من 4 ذكور مقسمون الى اربع مراحل عمريه.في نهاية فترة العلاج تم قياس تركيز هرمون التستوستيرون في الدم وتركيز هرمون البروجسترون بطريقه ELISA.واظهر اختلاف كبير في تراكيز هرمون التستوستيرون في الدم ولوحظ ذلك في مجموعة الذكور مقارنة مع مجموعة االسيطره. وقد أظهرت نتائج هذه الدراسة أن تأثير اوراق نبات الجرجير وخاصة في الجرعات العالية يمكن أن يزيد تركيز هرمون التستوستيرون في ذكور الإنسان.

مفتاح الكلمات: تقنيه الاليزا, اوراق الجرجير, الهورمونات الذكريه.

Introduction

Eruca sativa belongs to the family Brassicaceae. The scientific name is *Eruca sativa mill*, this family is one of the biggest families in plants kingdom because it contains a large group of a very important economic plants Garden rocket (*Eruca sativa Mill*.) it's grown in Europe mainly under shields, to obtain fresh, aromatic leaves. Nitrogen and potassium, as basic plant nutrients, are also indispensable elements in human nutrition (1,2,3). Since it has been used in traditional medicines as remedies for different diseases, therefore phytochemical composition and respective biological activities are important to understand the therapeutic potential of medicinal herbs. Nowadays,

there is an increasing focus for the search of natural source (non-synthetic)from medicinal plant such as carotenoids, ascorbic acid (vitamins),phenolic and flavonoids.(4)

Datura stramonium plant is effective against mammary gland adenocarcinoma ,(5).Another plant is *Seidlitzia rosemarinus* in which its extract has an antimicrobial effect against some pathogenic microorganisms causing vaginal infections .(6).

Eruca sativa content a large amount of minerals such as Ca , Fe, Mg, K, S, P, and it content a large group of vitamin E, C, K, I and also *Eruca* rich with Carotine pigment and vitamins like B1, B2, B6, B12.(7).

ELISA Systems has years of experience designing, testing, and optimizing immunoassay kits to ensure the highest level of performance in analyte quantification. Quantizing ELISA Kits provide specificity, accuracy, and sensitivity. (8)

Testosterone is a steroid hormone from the androgen group. Testosterone is primarily secreted in the testes of males and the ovaries of females although small amounts are secreted by the adrenal glands.(9)

It is the principal male sex hormone and an anabolic steroid. In both males and females, it plays key roles in health and well-being.(10).

Materials and Methods

1-Preparing the plant *Eruca sativa* leaves:

Eruca sativa leaves were obtained from local market in Baghdad City. The taxonomic identification of the plant was confirmed by a senior plant in the department of Botany, college of Science,University of Baghdad . *E. sativa* leaves were cleaned by washing under running water, *E. sativa* leaves were prepared by weighting 25,50,100 g of fresh *E. sativa* leaves respectively. Then, Kept in a refrigerator at 4C until use (10)

2- Preparing of Group samples

Male human were divided into Four groups of 4 male at different age period in each. Control group was given zero dose of plant.

3- Treatments

Eruca sativa leaves were freshly obtained before administration. Three doses of 25,50 and 100 g were given the group A,B,C respectively and D was the control group. Each group was give the dose for 30 days once time a day.

4-Measuring the concentration of hormones

After the experimental period(30 days) was end,5 ml of Blood were collected from each patients, serum was obtained by separated by centrifugation at 3000 rpm 15 minute and stored frozen at --20C further study.

5- (ELISA): Enzyme – linked immunosorbent assay (analysis):

Hormone analysis. Hormone concentrations were determined by use of the commercial human Uno ELISA kits (Immunolab GmbH, Kassel, Germany),according to the manufacturer's instructions. Standard solutions of 17β-estradiol, progesterone and testosterone (six concentration levels) and prepared solutions of plasma samples were instilled in the microwells. Then solutions of the dissolved enzyme conjugate (peroxidase), substrate (tetramethylbenzidine -TMB) and antibodies were added, with microplate incubation at room temperature in the dark. The microwells were washed in phases with the use of ELISA washer (ELx50, Bio-Tek Instruments,Germany). The reaction was stopped by the addition of stop solution (0.5 M sulphuric acid) and absorbance was measured on an ELISA reader at a wavelength of 450 nm (ELx800TM, Bio-Tek Instruments, Germany). Upon plotting the calibration curve, the serum hormone concentration was determined by use of the R-Biopharm Ridasoft Win software. Results were expressed in ng/mL (ppb) taking the plasma dilution factors into account.

6- Statistical Analysis:

Statistical significance was assessed by using least significant differences – LSD (T-test)

P – value < 0.05 was considered significance

Result and Discussion

Serum level of testosterone was significantly increase in treatment groups compared to the control (table1),while serum level of progesterone was significantly decrease in treatment group compared to the control (table2).In regard to testosterone concentration, the change was may prominent in the higher dose.

The testosterone, epididymis and other reproductive organs are structurally and physiologically dependents upon the testosterone and other androgens .Testosterone stimulates growth and secretary activity of the reproductive (11, 12, 13).

So a significant increase of these hormones in our study could increase the number and function of somatic and germinal cells of testosterone and in results increase the testosterone and epididymis weight.

Leaves of *E.sativa* may contain important secondary metabolite such as flavonoids, alkaloids, tannins, phenols ,caponius ,ascorbic acid and those are used as remedies of many

diseases and required in traditional medicines(14).These products has positively influenced reproductive functions (15).

Rocket plant (*Eruca sativa*) has gained greater importance as a vegetables and spice around the world, it is also to be an important chemoprotective plant (16).

There are no studies concerning the effect of these plants alone on testosterone and progesterone concentration in human. Therefore in this study we tried to investigate the effect of *Eruca sativa* on these hormones.

The increase and decrees in these hormones may be resulted from presence of some trace element's (Cr,Cu,Fe,Mn,and Zn) in the leaves of this plants (17).

Besides, the presence of some minerals especially Cu which activate the functions of several classes of the immune system particularly those involved in antibodies production could causes this effect (18, 19, 20).

Table (1) Detection of Testosterone hormones obtained from male human at different weight of *Eruca sativa* leaves.

Group age (Year)	*Eruca sativa* leaves			
	Group A 25.0 g	Group B 50.0 g	Group C 100.0 g	Group D (control)
25-30	4.80	5.60	6.70	4.20 *
30-35	4.30	5.50	5.60	1.80
35-40	3.80	5.50	5.60	1.30
40-45	1.30	1.80	3.10	0.40

*= Testosterone =ng/ml
**Normal value Testosterone hormone (0.6 – 3.1)ng/ml

Table (2) Detection of Progesterone hormones obtained from male human at different weight of *Eruca sativa* leaves.

Group age (Year)	*Eruca sativa* leaves			
	Group A 25.0 g	Group B 50.0 g	Group C 100.0 g	Group D (control)
25-30	0.30	0.20	0.10	0.5*
30-35	0.30	0.10	0.00	0.5
35-40	0.20	0.10	0.00	0.4
40-45	0.20	0.10	0.00	0.3

*= progesterone =ng/ml

**Normal value progesterone hormone (0.0 – 1.0)ng/ml

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