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# Effect of Pomegranate Juice and Fresh Leaves of *Eruca vesicaria* on Testosterone Hormone Level in Blood Serum of Male Rabbits

***Seeham Ali Qasim, Abeer Salih Ali**

Dept. Biology-Coll. Sci, Mustansiriyeh University, Iraq
Email: seehamalkafajy@uomustansiriyah.edu.iq

**ABSTRACT**

Plants have been used as a treatment for many diseases from ancient to our times, it has a great source of different drugs that used for our health benefits. This study assessed the effects of diluted *Punica granatum (P. granatum)* juice and *Eruca sativa (E. sativa)* fresh leaves on testosterone hormone level in male albino rabbits. Twenty-one rabbits their's weight between 1500 - 1800g, divided into 3 groups each group consisting of 7 rabbits for 30 days. Group1 as a control, group 2 received 500 ml of diluted Punica juice daily; group 3 received 350 g of fresh leaves of Eruca sativa. Blood samples were collected after and calibrated 15 and 30 days for testosterone hormone. Statistically significant increase in testosterone levels in group (2) (*P. granatum*) at day 15, 30 respectively with mean values (13.671±0.830 and 22.257±0.822) ng/ml when compared with the control mean value (9.771±0.368 and 9.500±0.490) ng/ml at same days. And also, there was a significant increase in testosterone levels in group (3) *E. vesicaria* with mean values (12.100± 0.551and 15.071±0.61) ng/ml at day 15 and 30 respectively compared with the control mean value (9.771±0.368 and 9.500±0.490) ng/ml at same days. a significant increase($p < 0.01$) appeared in testosterone level in group 2 on day (15,30) with mean values(13.671±0.830 and 22.257±0.822) ng/ml compared with group 3 mean values (12.100± 0.551and 15.071±0.61) ng/ml at same days. Increment testosterone levels were dependent on phytochemical compound that present in both plants .Punica Granatum and Eruca vesicaria could be used safely for treatment of sexual dysfunction due to the decrease in serum testosterone level, also Studies should be carried out to identify and isolate the active ingredients in those plants and to be extracted and used as a treatment of infertility. We also recommend that further observation and studying of the herbals and their positive effect that help to improve the public health.

**Keywords:** Jarjeer, Testosterone hormone, Fertility

**Correspondence**:
Seeham Ali Qasim
Dept. Biology-Coll. Sci, Mustansiriyeh University, Iraq
**Email**: seehamalkafajy@uomustansiriyah.edu.iq

## INTRODUCTION

Plants were known to play an important role in the health by their biological activity in variety of cultures worldwide (23). It has been used to facilitate fertility, through fertility-promoting properties and aphrodisiacal qualities Arabs had used herbal medicine to enhance sexual activity and raise libido (8).

*Eruca vesicaria*, it's a member of the Brassicaceae family (known as mustard family). It has acquired more importance as spices and salad vegetables, as consumption of this herb has been raised in Middle Eastern and Europeans. This species is known as Jarjeer in Arab states and rocket in native English-speaking States as exploited by local herbal people (7). It is obvious rich in elements such as calcium, iron, sodium, potassium, and magnesium. Also, this herb has several natural antioxidants like vitamins A, B, C, E, Beta-carotene, and dietary fiber, flavonoids and glucoseinolates (20). However, this plant has a variety of therapeutic and medicinal uses like suppression of tumor genesis, anti-ulcer and hepatoprotective activities. Jarjeer is noticed to use for stomach diseases, scurvy, and as a diuretic effect moreover, ethanolic extract of rocket seeds had a powerful renal protective activity. The leaves and seeds utilized to enhance sexual desire and to be as an aphrodisiac because the ability to catalyze the steroid production from the testis (19). *Punica granatum* was commonly known as (pomegranate) which belongs to the family Punicaceae. It's used widely as a traditional medicine because of its curative features. Many studies reported the useful effect of pomegranate on mans' health, pomegranate has proven to rise testosterone levels which have a main role in preservation of secondary sexual features and spermatogenesis. (18). Pomegranate is possessing a high level of polyphenol, tannins, and flavonoids like pedunculagin, punicalin, ellagic acid, and gallagic acid., it's also contains a high level of glucose, anthocyanins, ascorbic acid, caffeic acid catechin, numerous minerals, particularly amino acids and iron (6,15) .The main pharmacological functions assign to the extracts of pomegranate included anti-inflammatory, anti-hepatotoxicity, anti-diabetic anti-obesity , anticancer , antioxidants, antiviral, antibacterial and antifungal activities (13) . The current study aims to evaluate the beneficial effect of the juice of pomegranate and *Eruca vesicaria* leaves on the level of testosterone hormones in male rabbits that may make it one of the most important foods for the future.

## MATERIAL AND METHODS

### Plant samples

*Eruca sativa* and *Punica granatum* were bought from Baghdad local markets and identified by plant experts in Mustansiriyeh university herbarium.

### Plants preparation

### Pomegranate juice

A 2kg of (*P. granatum*) were cleaned and flaked by hand, without splitting the seeds. Then an electrical blender used to obtain the juice, filtered by Buchner funnel, diluted by DW to 1:3 volume daily for a month and 500 ml of the juice given to the first cage(2).

### *Eruca vesicaria*

350 g of fresh Jarjeer leaves were cleaned and prepared for the experiment.

### Experimental animals

Twenty-one adult male albino rabbits are used in this study their weighs is about 1.5 to 1.8 kg were used.

Standard laboratory conditions were maintained to the subjects throughout the study at room temperature of 25ºC to 30ºC with food and water. These Animals divided into three groups each group consisted of 7 rabbits.

**Group 1**: Control group: without any treatment.

**Group 2**: received 500 ml/day of diluted (*P. granatum*) juice orally for 30 days,

**Group3:** received by 350 g /day of (*E. sativa)* fresh leaves as food for 30 days.

Blood samples were collected in three stages, firstly in one day old of experiment, secondly in day 15 and third stage in day 30 breaching the heart after anesthetizing the rabbit, inserting the needle in an acute angle to obviate RBCs damage and breaching. Centrifuging the blood at 2000 g for 10 min. to separate the contact, then stored (at 4 °C) about 24 hours then used for hormone assay were obtained.

**Biochemical analysis**

The two groups were evaluated by measuring the level of hormone in the serum by chemoluminisence using VIDAS (bio-MerieuxSA) RCS LYON 673620399 Centaur system, which is an automated immunoassay analyzer.

**Statistical analysis**

The parameters values from the investigations were analyzed by SPSS (computer software) for variance (ANOVA) and T-test. The assessed expressions are the mean ± standard error, and differences between means. A comparison was made with the experimental control group. Differences were regarded statistically significant at the P≤ 0.05 and 0.01 level.

## RESULTS AND DISCUSSION

In(table1), The insignificant difference was detected between the testosterone hormone levels in group **2** (pomegranate juice) in one day old of experiment compared with control mean values (9.400±0.635), (9.657±0424) ng/ml respectively However, a significant increase in testosterone levels were observed ($p< 0.01$) in the group**2** on day 15 and day 30 with mean values (13.671±0.830), (22.257±0.822) ng/ml in comparing with the control values, (9.771±0.368), (9.500±0.490) ng/ml correspondingly .

In (Table **2**) There were no significant increasing in testosterone level in group **3** feeding with (*E. vesicaria*) at one day old of experiment compared with control (9.086±0.776), (9.657±0.424) ng/ml respectively while significant increase ($p<0.01$) was noticed in hormone level on day 15 and 30 (12.100±0.551), (15.071±0.61) ng/ml compared with control (9.771±0.368), (9.500±0.490) ng/ml respectively as detected. In(Table **3**),The differences between testosterone level in group **2** and group **3** on a day (1**,**15and30) were also determinate, as no significant increasing recorded in hormone level in group 2 compared with group **3** on one day old of experiment (9.400±0.635),(9.086± 0.776) ng/ml respectively Although, there was significant increase ($p < 0.01$) in the serum levels of testosterone in group**2** (pomegranates juice) after 15 days compared with group **3** feeding with (*E. vesicaria*) (13.671±0.830), (12.100± 0.551) ng/ml respectively Furthermore there was a significant increase in testosterone value in group **2** compared with group **3** at day 30 with value (22.257±0.822) and (15.071±0.61) ng/ml respectively.

**Table 1.** Total testosterone levels(ng/ml) of rabbits received (P.granatum juice) for 30 days.

| Group / day of experiment | N | Cont. group 1/ Testosterone value (ng/ml) Mean ± sd/sem | Group 2/ (Punica juice) Testosterone value (ng/ml) Mean ± sd/sem |
|---|---|---|---|
| 1 day | 7 | 9.400 ± 0.635 / 0.240 | 9.657 ± 0.424/ 0.160 |
| 15 day | 7 | 13.671 ± 0.830 / 0.314 | 9.771 ± 0.368 / 0.139 |
| 30 day | 7 | 22.257 ± 0.822 / 0.311 | 9.500 ± 0.490 / 0.185 |

**Table 2.** Total testosterone levels (ng/ml) of rabbits received (*E. vesicaria*) daily for 30 days

| Group / Day of experirment | N | Cont. group 1 Testosterone value (ng/ml) Mean ± sd/sem | Group 3 (E.sativa) Testosterone value (ng/ml) Mean ± sd/sem |
|---|---|---|---|
| 1 day | 7 | 9.657 ±0.424/ 0.160 | 9.086± 0.776 / 0.293 |
| 15 day | 7 | 9.771±0.368 / 0.139 | 12.100± 0.551 / 0.208 |
| 30 day | 7 | 9.500±0.490 / 0.185 | 15.071±0.61 / 0.233 |

**Table 3:** The Comparison between the testosteron level in each group 2 (*P. granatum*) and group 3 (*E. vesicaria*) with control in 30 day of receiving

| Group / Day of experiment | N | Cont. Testosterone value (ng/ml) Mean ± sd/sem | Group 1 (Punica juice) Testosterone value (ng/ml) Mean ± sd/sem | Group 2 (E.sativa) Testosterone value (ng/ml) Mean ± sd/sem | P value anova |
|---|---|---|---|---|---|
| 1 day | 7 | 9.657 ± 0.424/ 0.160 | 9.400 ± 0.635 / 0.240 | 9.086 ± 0.776 / 0.293 | 0.260 |
| 15 day | 7 | 9.771 ± 0.368 / 0.139 | 13.671 ± 0.830 / 0.314 | 12.100 ± 0.551 / 0.208 | 0.000 |
| 30 day | 7 | 9.500 ± 0.490 / 0.185 | 15.071 ± 0.61 / 0.233 | 22.257 ± 0.822 / 0.311 | 0.000 |
| p value | 0 | 0.000 | 0.000 | 0.000 | 0.000 |

*Punica Granatum and Eruca vesicaria* are plants that widely used because of their high nutritional value. The pomegranate juice comprises a large number of natural antioxidants, such as α-tocopherol, ascorbic acid and large polyphenols which include ellagitannins and punicalagins (22), these polyphenols pass through the small intestine and stomach pH without any changes also granatum contains a mix of polyphenolic anthocyanidins (12) . Dependence on the electron transfer system mitochondrial energy that is used to drive the synthesis of testosterone hormone exposed mitochondria of the Leydig cell to oxidative stress and when the testosterone hormone is stimulated, the oxidizing by-products formation increases inside Leydig cell (25), the effectiveness of oxidative stress on Leydig cells decrease activities of antioxidant enzymes, so the polyphenols with metabolites like - ellagic acid, punicalagins, Gallic acid, Anthocyanidins, urolithins have a strong antioxidant effectiveness, contain many binding sites for single free radical electrons that stimulate the testes to produce testosterone and makes the toxin-induced oxidative stress less (10). Expression of antioxidant activities commensurate with the number of its compound groups that makes the antioxidant capacity increase while the oxidative stress decrease in males (21). moreover pomegranate considered a good source of phenolic ,ellagic tannins, anthocyanins and other compounds which are already proved testosterone hormone by decrease the stress hormones (like cortisol), that seen by Hong *et al*. (14), this depending on a negative relation between cortisol hormone and testosterone synthesis, the levels of cortisol hormone increase inside the body The increment of the stress hormone (cortisol) push the body to pause the release of the main stimulating gonadotropin releasing hormone (GnRH), that's accountable for sperm count., and sexual activity (16) ,also the presence of the Zinc and vitamin C promotes hormone level and Fertility because of its activity as a good nutrient antioxidant (4) , While in group 3 *E. vesicaria* the increase in testosterone hormone level occurred because the presence of saponins , alkaloids and some extant flavonoids in rocket leaves (26) which are affected by opposition with enzymes that contribute in metabolism of testosterone such as aromatase and5-Alpha-reductase as well as Vitamin E. responsiveness of Leydig cell to the LH hormone (1). commensurate to Vitamin E magnitude of Vitamin E that is exposed by the cells and vitamin E supplemental bioavailability is high in human also is considered the most powerful breaking chain, lipid-soluble nutritional antioxidant Consistent with some reports the dietary supplement for 2 months of 483 mg/day of Vitamin E shown 20% increment of the synthesis of testosterone level in healthy males/ (11). Prevent the decline in Leydig and Sertoli cells number and increases them lead to increase in testosterone hormone level, prohibits reduction in a number of Sertoli and Leydig cells and increases them that lead to increases in testosterone hormone (17, 5), It also contains Zn, Cu, Fe, Mg, these elements supplementation may block the oxidative stress, which is caused by the suppression of antioxidant enzyme activity of the testis and synthesis of the testosterone (3). Moreover, some elements such as Mn which raise the immune response and reproductive efficiency (24,9). Both Punica Granatum and Eruca vesicaria could be used safely for treatment of sexual dysfunction due to the decrease in serum testosterone level, also Studies should be carried out to identify and isolate the active ingredients in those plants and to be extracted and used as a treatment of infertility. We also recommend that further observation and studying of the herbals and their effect on general health that help to improve the public health.

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