

## The Effect of Pomegranate Juice Extract on Hormonal Changes of Female Wistar Rats Caused by Polycystic Ovarian Syndrome

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### ABSTRACT

One of the important criteria in polycystic ovarian syndrome, found in investigations, is changes in sex hormone levels. Several therapies have been proposed till now for polycystic ovary disease. Due to the side effects of medications, it is critical to identify and provide alternative medicines. Considering the above-mentioned issues and the importance of therapy for these patients, so this study was performed with the purpose of investigation on the effect of pomegranate juice extract on changes of active hormones in patients with polycystic ovary syndrome. A total number of 56 female Wistar rats, weighing 170-200 g, were selected. They all had 2 to 3 consecutive estrous cycles during 12 to 14 days. The rats were then divided into 6 groups of 8 each that included: control 61 group (without getting any substances were kept for 61 days); PCOS61 group (they intramuscularly received 4 mg estradiol valerate and were killed after 61 days); control 81 group (without getting any substances were kept for 81 days); PCOS81 group (they intramuscularly received 4 mg estradiol valerate and were killed after 81 days); experimental 1 group (4 mg estradiol valerate through a single injection and 100 mg/kg Pomegranate extract by gavage); experimental 2 group (4 mg estradiol valerate through a single injection and 200 mg/kg Pomegranate extract by gavage); experimental 3 group (4 mg estradiol valerate through a single injection and 400 mg/kg Pomegranate extract by gavage). At the end of the study, mice of all groups were weighed and then they were made unconscious and blood samples were taken from them. After that, serum samples were obtained and free testosterone blood factor, estrogen and androstenedione were measured by ELISA. The results show that the concentration of estrogen and testosterone hormones and androstenedione in the 61- and 81-day experimental groups had a significant increase compared to the 61- and 81-day control group and in the experimental minimum, average and maximum groups has a significant reduction compared to the 61- and 81-day PCOS groups. Given the above-mentioned issues, the use of pomegranate juice improved changes in female sex hormones in PCOS patients. Therefore, it is recommended to use this extract to reduce the symptoms of polycystic ovary syndrome.

**Key words:** Pomegranate, sex hormones, PCOS syndrome, Rat.

## INTRODUCTION

Polycystic ovary syndrome was first introduced by Stein and Leventhal, in 1935<sup>1</sup>. This syndrome is a heterogeneous complex disorder which is affected by multiple factors. Approximately, 4 to 18 percent of women are affected by it and some complications like reproductive dysfunctions and metabolic and mental disorders arise in women with polycystic ovary syndrome (PCOS). The hypothalamic-pituitary-adrenal axis has a fundamental role in the growth and regulation of reproductive system in men and women. The activity of this axis has been reported high in patients with polycystic ovary syndrome<sup>2</sup>.

Studies show that a number of endocrine disorders in polycystic ovary syndrome strengthen and intensify each other<sup>3</sup>. Several therapies, such as changes in lifestyle, administration of medicine, and surgery, have been proposed till now for polycystic ovarian disease. Now the most recognized therapy is taking medications such as clomiphene citrate, metformin, letrozole, and tamoxifen<sup>3</sup>. Given to the side effects of these medicines, identification and synthesis of alternative medicines will have significant importance. There has been always a special attention to medicinal plants since ancient times and today, with numerous studies performed in this regard by the title of herbal medicine, worthwhile and beneficial effects of most of plants are obtained<sup>4</sup>.

Pomegranate by scientific name of *Punica granatum* is one of known fruits. Its cultivation in Iran and Middle East has a long history. Pomegranate plant grows in hot arid regions<sup>5</sup>. It has numerous medicinal properties and is mentioned as a paradise plant. Various parts of Pomegranate fruit contains different metabolites such as variety of vitamins such as B2, C, and B1, and folic acid, pantothenic acid, sugars, organic acids, alkaloids, polyphenols, and anthocyanins. Hydrolyzed tannins called *Punic alagins* constitute the most important polyphenols of pomegranate juice<sup>6, 7</sup>. Studies expressed that the red color of pomegranate juice is due to the presence of anthocyanins such as delphinidin, cyanidin, and pelargonidin glucoside<sup>8, 9</sup>. It was also stated in investigations that pomegranate seed oil is one of

the six known sources of saturated and unsaturated fatty acids<sup>10</sup>.

Pomegranate is an antioxidant-rich fruit (approximately as much as green tea) and contains a large amount of vitamin C and potassium. Past studies on pomegranate impact on male reproductive system showed that pomegranate juice increases the number of live birth in mice. Also stated that in addition to increase in live births, the fertility in male mice increased, as well<sup>11</sup>. Studies on aqueous extract of pomegranate seeds on the base activity of rat uterine smooth muscles indicated that this extract accumulatively and dose dependently increases contraction force in these muscles; it probably has an important role in reducing postpartum hemorrhage<sup>12</sup>. Considering the above mentioned issues, medicinal importance and pomegranate various effects on reproductive system, this study was performed by the purpose of investigation on the effect of pomegranate extract on hormonal changes caused by polycystic ovary syndrome in female Wistar rats.

## METHOD

This research was performed experimentally, completely randomized and in vitro condition. A number of 56 adult rats that no research had been done on them and/or on their parents, with 2 or 3 consecutive regular estrous cycle were bought from Jahrom University of Medical Science. Compressed foods produced by Animal Feeds Joint Stock Company used to feed the animals. The ambient temperature was adjusted at  $23 \pm 3$  °C, and the rats were kept in a 12-hour of light and 12-hour of darkness cycle. Throughout the test, the rats were supplied with water and special food for mouse, without any restriction.

To perform the test on adult female rats, they all should be placed on a sexual cycle. The rats were in different phases of estrous and an approach must be taken to place them all on one cycle. For this purpose, their vaginal smears were prepared to ensure that they were all in a same phase of serous cycle. Estradiol valerate (0.2 mL, at concentration of 4 mg/kg) was injected by syringe (manufactured by Razi Pharmaceutical Company) to induce ovarian multiple cysts. The rats in PCOS

groups received this amount intramuscularly and in a single injection.

To prepare pomegranate extract, the plant powdered at first with electrical mill, and soxhlet method used then for extraction. In this method, for every 10 grams of powdered pomegranate 200 ml of related solvent consisting of water and ethanol was added to it and the obtained mixture poured into the soxhlet device. Finally, the solvent separated from the extract using Rotavapor device.

The PCOS cases were randomly divided into 6 groups of 8 each:

**Control61 group:** without getting any substances the rats were kept for 61 days and in normal conditions.

**PCOS61 group:** the rats intramuscularly received 4 mg estradiol valerate in 0.2 ml sesame oil through a single injection and they were killed after 61 days.

**Control81 group:** without getting any substances the rats were kept for 81 days.

**PCOS81 group:** they intramuscularly received 4 mg estradiol valerate in 0.2 ml sesame oil through a single injection and were killed after 81 days.

**Experimental1 group:** this group received 4 mg estradiol valerate in 0.2 ml sesame oil through a single injection and 100 mg/kg pomegranate extract by gavage.

**Experimental2 group:** this group received 4 mg estradiol valerate in 0.2 ml sesame oil through a single injection and 200 mg/kg pomegranate extract by gavage. **Experimental3 group:** this group received 4 mg estradiol valerate in 0.2 ml sesame oil through a single injection and 400 mg/kg pomegranate extract by gavage.

After the end of the experiment, the rats anesthetized and placed on dissecting table. After that process blood samplings were obtained directly and carefully from their hearts and ventricle section using 5 CC syringe. Serum was prepared then and the concentration of estrogen, free testosterone, and andrestandion hormones were measured by ELISA method. Then to express the dispersion of a set of numbers, the amounts were given as SEMmean. One-way analysis of variance (one-way ANOVA) followed by Duncan test were used for comparison between groups and for multiple comparison between different groups, respectively. The obtained values were statistically considered significant at level of <P 0.05.

## RESULTS

Comparison of groups revealed that estrogen concentration in PCOS61 group and in PCOS81 group had significant increase compared with control 61 group, and control 61 and 81 groups, respectively. The experimental groups with consumption of minimum and average dose of the extract had significant increase compared with control 61 and control 81groups. The experimental groups with consumption of minimum, average, and maximum dose of the extract had significant reduction compared with PCOS61 and PCOS81 groups (Table 1).

Comparisons of groups revealed that the concentration of free testosterone hormone in PCOS61 group, and in PCOS81 group had significant increase compared with control 61

**Table 1: Comparison of different groups in terms of changes in estrogen hormone**

Parameter Groups	Estrogen Hormone (ng/dl)
Control 61	67.983±2.287a
PCOS61	222.4831.969d
Control 81	67.3333.242a
PCOS81	228.3001.617d
Experimental 1	182.6003.762c
Experimental 2	113.2004.079b
Experimental 3	60.000 1.702 a

**Table 2: Comparison of different groups in terms of changes in free testosterone hormone**

Parameter Groups	Free Testosterone Hormone (ng/dl)
Control 61	0.11±0.010a
PCOS61	3.3560.0886d
Control 81	0.1330.008a
PCOS81	3.8230.321e
Experimental 1	1.5480.092c
Experimental 2	1.1760.037c
Experimental 3	0.5460.026b

group, and control 61 and control 81 groups, respectively. The experimental groups with consumption of minimum, average, and maximum dose of the extract had significant increase compared with control 61 and control 81 groups. The experimental groups with consumption of minimum, average, and maximum dose of the extract had significant reduction compared with PCOS61 and PCOS81 groups (Table 2).

The obtained results from measurement of andrestandion hormone between different groups revealed that the concentration of this hormone in PCOS61 group, and in PCOS81 group had significant increase compared with control61 group, and control 81 groups, respectively. The experimental groups with consumption of minimum, average, and maximum dose of the extract had significant decrease compared with PCOS61 and PCOS81 groups (Table 3).

### DISCUSSION

The obtained results showed that there were significant increases in related changes to estrogen, free testosterone, and andrestandion (androgen) hormones in PCOS61 and PCOS81 groups compared with control 61 and control 81 groups, respectively. Women with polycystic ovary syndrome have disorders in androgens metabolism and estrogen synthesis<sup>13</sup>. Clearly, in

this illness women are faced with reduced amount of progesterone hormone, and with increased amount of estradiol, testosterone, and andrestandion hormones. Depending on stimulation level of LH hormone in women with PCOS, the daily means of estrogen and androgen synthesis increase<sup>14</sup>. As reported in investigations, the symptoms of this syndrome are hirsutism and acne due to excessive synthesis of androgen hormone<sup>1</sup>. It was also indicted that polycystic ovary syndrome is associated with many endocrine disorders. Among them can mention to impairment in secretion of gonadotropins, especially LH hormone. Increased levels of LH will raise the secretion of androgen hormone<sup>3</sup>. Other studies expressed that insulin and insulin-like growth factors (IGFs) increase in women with PCOS which in turn will raise androgen synthesis in single cells<sup>3</sup>. This result is consistent with the present study. Other studies reported that polycystic ovary syndrome increases secretion of testosterone and LH hormones and reduces FSH hormone<sup>15</sup>.

Studies reported about the amount of secretion of testosterone and andrestandion hormones that mutation in aromatase P450 enzyme in ovarian tissue resulting in impaired function of these enzymes. It also increases androgens which are probably due to this syndrome<sup>16</sup>. According to the past studies it was stated that aromatase has been successfully used for induction of ovulation. It is likely that women with polycystic ovary syndrome have relative deficiency of aromatase in their ovaries, which leads to the increased level of ovarian androgens and eventually leads to creation of multiple small follicles (polycystic ovarian morphology)<sup>17</sup>. Androgens also increase FSH receptors synthesis in women with PCOS and these patients have high sensitivity to increased level of FSH in response to exogenous gonadotropins. So, increased FSH in women with PCOS could raise the risk of ovarian hyperstimulation syndrome and multiple ovulating<sup>17</sup>.

Presumably increase of estrogen, testosterone, and andrestandion hormones in this study can also be due to mutation in aromatase P450 enzyme that catalyzes biosynthesis of estrogen from androgen. The results of this research are similar to past findings in polycystic women.

**Table 3: Comparison of different groups in terms of changes in andrestandion hormone**

Parameter Groups	Andrestandion Hormone (ng/dl)
Control 61	0.080±0.004a
PCOS61	0.5600.015d
Control 81	0.0840.004a
PCOS81	0.5720.023d
Experimental 1	0.2200.003c
Experimental 2	0.1940.010c
Experimental 3	0.1340.009b

\* The available means in each row which at least has one letter in common does not have significant difference at level of 5% of Duncan test.

The concentration of estrogen, free testosterone, and androstenedione hormones in the patient groups receiving pomegranate extract, in this study, has significant decrease compared with the patient control groups. This is due to the protective effect of the extract and the protection effect becomes more sensible by increasing dose of pomegranate extract. It shows and confirms positive effects of this extract on reduction of hormonal complications of polycystic ovary syndrome.

The reason which can be stated for decrease of hormones is that phytosterols are substances that are naturally available in plants and these are similar to cholesterol that can interfere in absorption of cholesterol, can reduce the amount of low-density lipoprotein (LDL) that sometimes referred to bad cholesterol and triglycerides, and play role in reduction of androgen and particularly testosterone hormones<sup>18</sup>. It was also stated in another research that available phytoestrogens in herbal extracts reduce synthesis of gonadal steroids such as testosterone through reduction of cholesterol<sup>19</sup>, which agrees with findings of this study.

Other performed investigations on compounds containing phytoestrogens showed that long-term use of these compounds will reduce the amount of testosterone hormone<sup>20</sup>. Studies indicated the reduced amount of testosterone in rats fed with phytosterol for 22 days compared with control group<sup>21</sup>, which is consistent with findings of this study.

Studies showed that pomegranate seed oil contains phytoestrogen which prevents from appearance of cancer<sup>22</sup>. In plants containing phenolic compounds similar to pomegranate extract, were also found that the concentration of estradiol hormone in patients with polycystic ovary syndrome has significant reduction than control group, which indicates the positive effects of phenolic compounds in improving complications of polycystic ovary syndrome<sup>23</sup>.

Study on phenolic compounds revealed that these compounds have anti-androgenic properties and apply their inhibitory effect on formation of dihydrotestosterone receptor complex. They also reduce secretion of testosterone hormone<sup>24</sup>. Various studies revealed that these plants have phenolic compounds<sup>25, 26</sup>.

Therefore, that phenolic compounds of pomegranate extract lead to reduced effect of testosterone hormone through inhibition from formation of dihydrotestosterone receptor complex is also a possibility that discussed in the present study. Remission can be justified by this effect based on the reduction of testosterone effect. In addition, the extract may enhance the activity of aromatase enzyme, and cause reduction in testosterone levels through aromatization process and by conversion of testosterone to estrogen. It is expected thus estrogen concentration increases, but the results does not reveal such variation. Reduced amount of estrogen hormone can also be for the reason that all of steroid hormones such as estradiol are derived from cholesterol. As a result, synthesis of steroid hormones such as estradiol decreases by reduction of cholesterol concentration<sup>19</sup>. According to researches, feeding with pomegranate seed oil significantly reduces the accumulation of triglycerides and unsaturated fatty acids in rats<sup>22</sup>. So it is likely that in spite of conversion of testosterone into estrogen, the cause that the concentration of estrogen is still low in groups receiving the extract in the study is an issue that pomegranate extract reduces body fat and cholesterol reserves that are effective in concentration of estrogen hormone.

## CONCLUSION

According to the above, consumption of pomegranate extract improves changes of female sex hormones by reducing the concentration of estrogen, free testosterone, and androstenedione hormones in patients with PCOS. So, consumption of the extract is recommended for reduction of polycystic ovary syndrome complications.

## REFERENCES

1. Abedian N, Madani T. The effect of Ramadan fasting on hypothalamic-pituitary-adrenal axis in patients with polycystic ovary syndrome. *Iran's Journal of Obstetrics and Gynecology and Infertility*. **16**(57): 7-16 (2013).
2. Legro RS. Polycystic ovary syndrome and cardiovascular disease: a premature association? *Endocrine reviews*; **24**(3):302-12 (2003).
3. Marx TL, Mehta AE. Polycystic ovary syndrome: pathogenesis and treatment over the short and long term. *Cleveland Clinic journal of medicine*. ; **70**(1):31-3 (2003).
4. Amirghofran Z. Medicinal plants as immunosuppressive agents in traditional Iranian medicine. *Iranian journal of immunology: IJI*. **7**(2):65-73 (2010).
5. Sarkhosh A, Zamani Z, Fatahi R, Ghorbani H and Hadian J. A review on medicinal characteristics of pomegranate (*Punica granatum* L.). *J. Med. Plants*; **6**(22): 13 - 24 (2007).
6. Poyrazog E, Knew W and Artik. N. Organic acids and phenolic compounds in Pomegranates (*Punica granatum* L.) grown in Turkey. *J. Food Compos. and Anal.*; **15**: 567 - 75 (2002).
7. Malgrejo P, Salazar, D and Arties F. Organic acids and sugars composition of harvested pomegranate fruits. *Euro. Food Res. Technol.* **211**: 185 – 90 (2000).
8. Young Lim Kho, Woong Jung, Dohyeong Kwon and Jung Hoan Kim. Identification of estrone in pomegranate (*Punica granatum*) extracts by liquid chromatography-tandem mass spectrometry. *Food Science and Biotechnology*, **19**(3): 809-813 (2010).
9. Don Woong Choi, Jee Yeun Kim, Seon Hee Choi, Hee Su Jung, Hyo Joo Kim, So Yean Cho, Chan Soon Kang and Seung Yeup Chang. Identification of steroid hormones in pomegranate (*Punica granatum*) using HPLC and GC–mass spectrometry. *Food Chemistry*, **96**(4): 562–571 (2006).
10. Hernández-Valencia M, Hernández-Rosas M, Zárate A. [Care of insulin resistance in polycystic ovary syndrome] [Article in Spanish]. *Ginecol Obstet Mex*; **78**(11):612-6 (2010).
11. Amini Rad A, Khalili M.A, Soltani Gard Faramarzi H.R. The effect of pomegranate juice consumption on sperm parameters and fertility potential in mice. *Hormozgan Medical Journal*, **13**(3): 182-188 (2009).
12. Nouroldini M, Namdar F, Hydari A, et al. The effect of aqueous extraction of pomegranate seeds on base activity of uterine smooth muscle of virgin rats. *Journal of Physiology and Pharmacology*, **17**(4): 461-468 (2013).
13. Yildirim B, Sabir N, Kaleli B. Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. *Fertility and sterility*; **79**(6):1358-64 (2003).
14. Calogera AE, Macchi M, Montanini V, Mongioi A, Maugeri G, Vicari E, et al. Dynamics of Plasma Gonadotropin and Sex Steroid Release in Polycystic Ovarian Disease After Pituitary Ovarian Inhibition with an Analog of Gonadotropin-Releasing Hormone\*. *The Journal of Clinical Endocrinology & Metabolism*; **64**(5):980-5 (1987).
15. Karam Pour P, Azar Nia M, Abolghasemi GH, et al. The impact of fennel seed hydroalcoholic extract on serum levels of sex hormones in female Wistar rats with polycystic ovary syndrome. *Journal of Scientific Research of Arak University of Medical Sciences*. **17**(5): 70-78 (2014).
16. Forster H, Niklas H, Lutz S. Antispasmodic effects of some medicinal plants. *Planta medica*. **40**(12):309-19 (1980).
17. Requena A, Herrero J, Landeras J, Navarro E, Neyro JL, Salvador C, et al. Use of letrozole in assisted reproduction: a systematic review and meta-analysis. *Hum Reprod Update* **14**: 571-82 (2008).
18. Sugano M, Kamao F, Ikeda I, Morik H. Lipid-Lowering of phytostands in rats. *Atherosclerosis*; **24**:301-39 (1996).
19. The effects of *White Horehound* (*Marrubium Vulgare*) hydroalcoholic extract on parameters of polycystic ovary syndrome in adult female rats. *Journal of Medical Science*

- of Islamic Azad University*, **24**(2): 74-80 (2014).
20. Nasri S, Oryan Sh, Haeri Rohani A, Amin GH, Taghizadeh M. The effects of *Vitex agnus castus* L. extract and interaction with bromocriptine on luteinizing hormone and testosterone in male mice. *Medical journal of Hormozgan university*. **9**:113-8 (2005). [Persian]
  21. Awad AB, Hartati MS, Fink CS. Phytosterol feeding induces alteration in testosterone metabolism in rat tissues. *J Nutr Biochem*; **9**:712-17 (1998).
  22. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, Hayek T, Presser D and Fuhrman B: Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E deficient mice. *Am. J. Clin. Nutr*; **71**: 1062 - 76 (2000).
  23. Milewicz A, Gejdel E, Sworen H, Sienkiewicz K, Jedrzejak J, Teucher T, Schmitz H, vitex agnus castus extracted in the treatment of luteal phase defect due to latent hyperprolactinemia. *Arzneimittelforschung* **43**: 752-756 (1993).
  24. Pathak V, Shirota O, Sekita S, Hirayama Y, Hakamata Y, Hayashi T, et al. Antiandrogenic phenolic constituents from *Dalbergia cochinchinensis*. *Phytochemistry*. **46**(7): 1219-23 (1997).
  25. Varasteh F, Arzani K, Zamani Z. A. Investigation on Physicochemical changes of Saveh sweet-and-sour pomegranate fruits (*Punica granatum*). *Iran's Journal of Horticultural Science (Iran's Agricultural Science)*. 2010; **39**(1): 29-38.
  26. Jamali H, Najafian M, Parhizgar M and et al. Antioxidant Effects of Ginseng Root Extract Against Side Effects of Azathioprine on the Rat Ovary. *World Journal of Zoology*; **8**(4): 425-431 (2013).