

Pomegranate Juice Repairs Sperm Abnormalities in Experimental Animals

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Abstract

Pomegranate fruit (*Punica granatum*) has been consumed as a natural drug, especially in the Middle East, because of its health benefits. Pomegranate fruit has marked antioxidant activity and high levels of polyphenols compared to other fruits. Pomegranate juice (PJ) prevents the damage during spermatogenesis. The present study investigates pomegranate juice positive affection on reproductive system, oxidation and antioxidant activity. The study has been conducted on adult male mice at the department of physiology, College of Veterinary Medicine, Al-Qadisiya University during the period extended from October to April 2021. Sixty mature male mice (aged 90 days and weighted 25 ± 10 g) were randomly assigned to 6 equal groups (10 each), control (C) males were drenched with drinking water daily for 60 days, Methotrexate treated males were drenched with suspension (mg/ kg b.w) daily for 35 and 60 days. Pomegranate juice treated males were drenched with suspension (50 mg/ kg b.w) daily for 20, 35 and 60 days. Methotrexate + Pomegranate juice (50 mg/ kg b.w) treated males were drenched with suspension daily for 35 and 60 days. Methotrexate + Pomegranate juice (100 mg/ kg b.w) treated males were drenched with suspension daily for 20, 35 and 60 days. Methotrexate + Pomegranate juice (200 mg/ kg b.w) treated males were drenched with suspension daily for 35 and 60 days. The present study showed a significant increase in serum testosterone concentrations after 20, 35, and 60 days compared with control. Epididymis samples obtained from Pomegranate juice treated group increases significant whither sperm status at 20-, 35-, and 60-day periods compared with control. In comparison between periods, a significant difference between periods and control group was recorded whereas at 20, 35, and 60 day periods compared with control treated group a significant increase in sperm status as the treatment period proceeded at the days 20th, 35th, and 6. In conclusion the Pomegranate juice antioxidant activity is of pharmacological value not only as an antioxidant but also as an inducer of endogenous enzymatic and non-enzymatic antioxidants by improving reproductive system expressed as levels of testosterone hormone and sperm status activity.

Keywords: Pomegranate, Methotrexate, testosterone, spermatogenesis

1. Introduction

Pomegranate fruit (*Punica granatum*) is considered as a natural medicine, worldwide, because of its health benefits [1-2]. Pomegranate fruit has marked antioxidant activity and high levels of polyphenols compared to other fruits [3-4]. Pomegranate juice (PJ) has been reported to prevent damage during spermatogenesis [2]. Reactive oxygen species (ROS) formed by Methotrexate (MTX) might be accountable for decreased semen quality [5]. Thus, the compounds in PJ, such as polyphenols, flavonoids, and anthocyanins, are considered strong antioxidants that scavenge ROS [6-7]. Methotrexate (MTX), an anticancer drug extensively utilized in the remedy of different sicknesses which including psoriasis and positive inflammatory sicknesses by interfering with mobile reproduction [8]. MTX is a chemical agent inhibits the enzyme dihydrofolic acid reductase, which catalyzes the conversion of folic acid into an energetic shape called folinic acid via binding [9].

A review has stated incidences of methotrexate toxicity inside the testicular tissue of mice uncovered to methotrexate. This review discovered that Methotrexate ends in impairment of male reproductive organs; subsequently the reversal of its

toxicity is vital due to its healing potentials [10]. However, no proof of pomegranate fruit and/or its extract effect on male fertility yet. Thus, the current study demonstrates the toxicity of MTX on the level of testosterone hormone and changes in testicular tissues, in addition to the effectiveness of PJ on reducing the effects of MTX.

2. Materials and Methods

Sixty mature male mice aged 90 days weighting 25 ± 10 g were assigned to 6 equal groups, control (C) males were given drinking water daily for 60 days, Methotrexate treated males were fed with suspension (50 ml/ kg b.w) daily for 35 and 60 days. Pomegranate juice treated males were fed with suspension (50 mg/ kg b.w) daily for 20, 35 and 60 days. Methotrexate + Pomegranate juice (50 ml/ kg b.w) treated males were fed with suspension daily for 35 and 60 days. Methotrexate + Pomegranate juice (100 ml/ kg b.w) treated males were fed with suspension daily for 20, 35 and 60 days. Methotrexate + Pomegranate juice (200 ml/ kg b.w) treated males were drenched with suspension daily for 35 and 60 days. Blood samples were drawn from abdominal vein using non-heparinized tubes for assessment of testosterone concentrations.

3. Results

3.1. Effect of Methotrexate, Pomegranate Juice and their Interaction on Testosterone Hormone

The level of testosterone in the MTX group at time periods of 20, 35, and 60 days, decreased significantly, compared to the control group for the same time periods (Table 1). Meanwhile, the results of the study show no-significant increase in the level of the testosterone hormone in the group treated with PJ only at the time periods of 20, 35, and 60 days compared to the control group and the MTX group for the same periods. The results of the current

study also indicate a significant increase in the level of the testosterone hormone after giving PJ at a dose of 50 ml with the same dose of MTX for the same time periods compared with the drug group MTX only for the same periods.

An increase in the level of the testosterone hormone occurred after giving pomegranate juice at a dose of 100 mg with the same dose of MTX for the same time periods compared with group MTX only for the same periods. The level of the testosterone hormone increased significantly after giving PJ at a dose of 200 ml with the same dose of MTX for the same time periods compared with the drug group MTX only for the same periods.

Table (1). The effect of methotrexate, pomegranate juice and their interaction on testosterone hormone (nmol/L) after treatment for three-time interval

Groups	Time (days)			p. value	LSD
	20	35	60		
Control	14.89±0.37 E/a	14.89±0.37 D/a	14.89±0.37 C/a	1.0	Non-sig
Methotrexate	6.89±0.59 A/c	5.10±0.21 A/b	3.69±0.35 A/a	< 0.001	0.64
Pomegranate juice	17.17±0.80 F/a	17.15±0.28 E/a	18.31±0.59 E/b	0.038	0.93
Methotrexate + Pomegranate juice 50 ml	9.25±0.24 B/a	10.59±0.45 B/b	12.24±0.38 B/c	< 0.001	0.57
Methotrexate + Pomegranate juice 100 ml	10.81±0.30 C/a	12.32±0.60 C/b	14.22±0.24 C/c	< 0.001	0.64
Methotrexate + Pomegranate juice 200 ml	12.79±0.24 D/a	14.38±0.48 D/b	15.62±0.46 D/c	< 0.001	0.64
p. value	< 0.001	< 0.001	< 0.001		
LSD _{0.05}	0.709	0.626	0.618		

1. The capital letters denote the comparison within the same column, while the small letters denote the comparison within the same row.
 2. LSD, least significant difference, different letters denote to significant difference at P≤0.05
- #### 3. 2. Sperm Motility (%)

Results exhibited no significant differences at (P≤0.05) in all study groups and for all different time periods compared with the control group (Table 2). Additionally, no significant decrease in the sperm motility (%) in the MTX group was recorded at the time periods of 20, 35, and 60 days compared to the control group for the same time periods. No-significant increase in the sperm motility (%) occurred in the group fed with PJ only an the time periods of 20, 35, and 60 days

compared to the control group and the MTX group for the same time periods.

A significant increase in the sperm motility (%) was recorded after feeding with PJ at a dose of 50 ml with the same dose of MTX for the same time periods compared with the drug group MTX only at the same periods. The results of the current study also indicate a significant increase in the sperm motility (%) after giving PJ at a dose of 100 ml with the same dose of MTX for the same time periods compared with the drug group MTX only and for the same periods. Meanwhile, a significant increase in the sperm motility was recorded (%) after feeding with PJ at a dose of 200 ml with the same dose of MTX for the same time periods compared with the drug group MTX only and for the same periods.

Table (2). The effect of methotrexate, pomegranate juice and their interaction at three time periods on sperm motility

Groups	Time (days)			p. value	LSD
	20	35	60		
Control	75.87±1.31 C/a	75.87±1.31 B/a	75.87±1.31 B/a	1.0	Non-sig
Methotrexate	95.9±1.31 E/a	98.65±0.89 E/a	99.68±0.32 E/a	0.001	1.45
Pomegranate juice	45.69±1.31 A/c	35.69±1.31 A/b	25.69±1.31 A/a	< 0.001	2.04
Methotrexate + Pomegranate juice 50 ml	65.99±1.28 B/a	75.99±1.28 B/b	85.99±1.28 C/c	< 0.001	2.00
Methotrexate + Pomegranate juice 100 ml	75.99±1.28 C/a	85.99±1.28 C/b	95.99±1.28 D/c	< 0.001	2.01
Methotrexate + Pomegranate juice 200 ml	85.9±1.28 D/a	95.91±1.21 D/b	99.24±0.49 E/c	< 0.001	1.69
P. value	< 0.001	< 0.001	< 0.001		
LSD _{0.05}	1.93	1.84	1.61		

3.3. Sperm counting (million/ml)

A significant decrease in the sperm counting (million/ml) occurred in the MTX group at the time periods of 20, 35, and 60 days compared to

the control group and for the same time periods (Table 3). On the other hand, a significant increase in the sperm counting was exhibited in the pomegranate juice group only at the time periods of 20, 35, and 60 days compared to the

control group and the MTX group for the same time periods. A significant increase in the sperm counting was recorded after giving PJ at a dose of 50 ml with the same dose of MTX for the same time periods compared with the drug group MTX only for the same periods.

There was a significant increase in the sperm counting (million/ml) after giving pomegranate

juice at a dose of 100 ml with the same dose of the drug MTX for the same time periods compared with the drug group MTX only for the same periods. Meanwhile, a significant decrease in the sperm counting occurred after feeding with PJ at a dose of 200 ml with the same dose of MTX for the same time periods compared with the drug group MTX only at the same periods.

Table (3). The effect of methotrexate, pomegranate juice and their interaction at three time periods on sperm counting (million/ml).

Groups	Time (days)			p. value	LSD
	20	35	60		
Control	62.02±1.4 C/a	62.02±1.4 B/a	62.02±1.4 B/a	1.0	Non-sig
Methotrexate	82.14±1.37 E/a	86.14±1.37 D/b	90.64±1.51 E/c	< 0.001	2.21
Pomegranate juice	32.19±1.3 A/c	22.19±1.30 A/b	19.19±1.28 A/a	< 0.001	2.02
Methotrexate + Pomegranate juice 50 ml	52.19±1.29 B/a	62.41±1.29 B/b	72.41±1.29 C/c	< 0.001	2.01
Methotrexate + Pomegranate juice 100 ml	62.41±1.29 C/a	72.41±1.29 C/b	82.41±1.29 D/c	< 0.001	2.01
Methotrexate + Pomegranate juice 200 ml	72.41±1.29 D/a	87.41±5.28 D/b	96.91±0.80 F/c	< 0.001	4.94
p. value	< 0.001	< 0.001	< 0.001		
LSD _{0.05}	1.97	3.68	1.90		

4. Discussion

Generally, low serum testosterone, also known as hypogonadism, which is documented to cause many signs and symptoms, including loss of libido, erectile dysfunction, diminished cognitive function, depression, lethargy, osteoporosis, muscle mass and strength, depressive symptoms, fractures, and mortality [1]. However, new studies have shown that men with low testosterone levels are more likely to die of coronary artery disease and stroke if they do not receive treatment [11]. Low levels of testosterone are observed in an animal group treated with MTX suggesting that the drug leads to adverse effect on the testes of the animals leading to malfunctioning in the production and release of the important sex hormone [9]. It is interesting to note that just about three weeks after exposure to the drug, the level of the hormone production in the treated animals had dropped more than 50% compared with the control (Table 1). This is an indication that the drug actually had a deleterious effect on the production of the hormone which may lead to disorders in sexual activity of the animal. Similarly, there were significant reductions in the level of the hormone in the group treated with the drug after 35 days and 60 days recorded 34.3 and 24.9% respectively. This suggests that the effect of the drug if not addressed, may lead to progressive deterioration in the animal physiological function particularly the sexual functions.

To our knowledge, the current findings support previous research that has shown that MTX drug cause adverse effect on the tested experimental animals [12], which is in accordance with our findings (Table 2). Testicle weight loss may be caused by a decrease in the number of seminiferous germ cells, Leydig cell lysis, and a drop in sperm production [13]. As the Leydig cells die, testosterone levels fall. In addition to impairing sperm maturation and quality, low testosterone concentrations are linked to

unfavorable effects on epididymal tissue function and therefore alter spermatogenesis. A decrease in testicular size, seminiferous tubule diameter, epithelial thickness, or increased interstitial space may also be associated with this weight loss, resulting in testicle destruction as has been reported [8].

The majority of the serum testosterone in male animals [9] is synthesized by the Leydig cells of the testis under the control of luteinizing hormone which is released from pituitary gland. Thus, defects whether acquired or congenital, interfere with the testis production of testosterone or interact with the hypothalamic-pituitary-gonadal axis (HPGA) can decrease testosterone level. Therefore, MTX may have influenced the pituitary gland by preventing the release of luteinizing hormone in the animals thereby causing a low level of the testosterone hormone production in testis as has been speculated recently [3]. Low testosterone levels may due to injury such as trauma, castration, radiation or chemotherapy, hormonal disorders like pituitary tumors or diseases, high levels of prolactin, or other diseases like mumps. Hence, medications have been implicated in low production of testosterone [14]. Methotrexate may be categorized among the drugs that negatively affects the secretion of testosterone in mammals as shown in this study (Table 1).

On the contrast, the group administered with PJ only, recorded a higher production of the testosterone hormone than the control with an increase reached up to 15.31% after 20 days of administration which later increased to 22.97% at day 60 (Table 1). This may due to that the PJ contains bioactive substances elicit the production of the hormone. Moreover, the observed increase in the hormone produced in the animals treated with varying concentrations of PJ mixed with MTX led to potential ability of the juice to ameliorate the negative effect of the MTX on the production of testosterone in the experimental animals.

It is interesting to note that the adverse effect of MTX

was amended in animals treated with 200 ml of the PJ after 35 days of exposure whereas, it took 60 days for those treated with 100 ml of the juice to fully recover to the normal level of hormone production. This result supports the earlier claim of the medicinal use of the PJ in the treatment of several ailments including sexual disorders [15]. It may also be linked with the presence of phytoestrogens in the fruit of this plant as reported recently [16] who suggested that endogenous estrogens compete with phytoestrogens for binding to the estrogen receptor, which reduces the hormonal action of phytoestrogens, which are structurally similar to estradiol, a steroid hormone.

Sertoli cells in the testis produce an important endocrine hormone called inhibin. Hormone production is regulated by a negative feedback impact on pituitary FSH levels, reducing spermatogenesis. It has been reported [17] that various variables can also affect the hypothalamus-pituitary-gonad axis, compromising regulation and balance. Pomegranate fruit components are rich in chemicals with a wide range of medicinal uses. The negative effects caused by MTX drug, appears to inhibit parasite development in the host prior to the formation and release of oocysts that causes the damage. As demonstrated herein, a considerable repairing agent in the infected mice reproduction tissues which is an evidence after treatment with PJ. Pomegranate fruit juice displayed antioxidant activity [18] by reducing the oocyst discharge in mice. Promising findings [3] showed that PJ has a substantial protection against tissue damage caused by *Eimeria* and that it should be used as a food supplement to improve the damaged testicles.

Several new treatments for damaged sperms and tissues have been developed; however, they alleviate symptoms and affect the reproductive organs. Furthermore, experimental mice with testicle damage may be unable to withstand the side effects of long-term usage of these medications [19]. Therefore, pomegranate fruit juice cures the damage caused by MTX drug. Recent research has focused on the possible potential effects of naturally occurring phytochemicals with powerful antioxidant properties in different plants for the treatment of damaged tissues and sperms in both human and animals [9]. The antioxidant properties of pomegranate may help in protecting neurons from the neurotoxic effects of MTX drug.

According to the current results, pomegranate fruit juice is safe as a therapeutic at the tested dosages because it has no harmful effects on both human and animal. Pomegranate fruit juice bioactive ingredients, including phenolic acids, flavonoids, tannins, and lignans, have been studied extensively [20], but little is known about their bioavailability, bio distribution, and metabolism according [21]. Pomegranate polyphenol bioavailability may be impacted by various factors, including individual variability and the processing of PJ and the analytical techniques used to identify low postprandial

quantities.

Pomegranate fruit juice has anti-oxidative and anti-lipid peroxidation properties, which may improve the sperm count and shape while MTX drug destroyed germ cells in the experimental mice. This study showed statistically significant differences in sperm count and no motile sperm characteristics (Table 3). An improvement may be achieved by combining the synergistic effects of pomegranate fruit juice with some other antioxidant plant extracts, which can have positive effects on the reproductive system [14]. The observed reduction in the sperm motility in the group given the pomegranate juice over 60 days' experimental period suggests a possible multidimensional effect of the juice on the sperm cells. On the other hand, the juice may contain certain components which may have interacted with the sperm cells in such a way that impede movement of the cells.

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Declaration: The authors confirm that no conflicting interest and all experimental work was carried out according to the valid legislations.