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A Study on the Effects of *Walnut oil* on Plasma Levels of Testosterone Pre and Post Puberty in Male Rats

Motahareh Bostani[1], Haidar Aqababa*[1], Seyed Ebrahim Hosseini[2] and Saeed Changizi Ashtiyani[3]

[1]Department of Physiology, Faculty of Sciences, Islamic Azad University, Arsanjan / Iran
[2]Assistant professor, Department of Physiology, Faculty of Sciences, Islamic Azad University, Fars Science and Research Branch, Iran
[3]Associate professor, Department of Physiology, Arak University of Medical Sciences, Arak / Iran

ABSTRACT

Background: As there is little knowledge about the impact of walnut oil on the physiology of the animals' reproductive system ,and considering the fact that walnut oil contains a high proportion of some important unsaturated fatty acids including alpha-linolenic acid (ALA) and Omega-3 , the present study aimed to investigate the possible effects of walnut oil on plasma levels of testosterone pre and post puberty in male wistar rats.
Materials and Methods: In this experimental study, 48 male wistar rats were randomly divided into two groups of pre and post puberty. Then each group was divided into three sub groups of control, sham and experimental, each of which with 8 animals. Walnut oil was administrated by gavage at a dose of 20 mg/ kg daily at the specified time (10 am) for 30 days. Blood samples were obtained by cutting off the tail and plasma levels were measured by using a radioimmunoassay method (RIA).
Results: In the groups receiving walnut oil in the pre-puberty period, testosterone levels significantly increased ($P < 0.05$). In the groups receiving walnut oil post puberty, testosterone levels significantly increased, too ($P < 0.01$).A significant difference between pre puberty and post puberty groups was observed. ($P < 0.001$)
Conclusion: This study showed that walnut oil had stimulating effects on the male reproductive system and could increase plasma testosterone levels by influencing the pituitary - testicular axis.

Keywords- Walnut oil, Testosterone, Male rats.

INTRODUCTION

Walnut is the oldest tree food known to man with a longevity extended to thousands of years. Walnut originated in ancient Persia. That is why it is often referred to as the Persian walnut. Walnut contains significant amounts of antioxidants, omega-3 fatty acids and vitamin E, minerals, iron, sodium, calcium, magnesium, manganese, copper, potassium, phosphorus, and also protein and fiber, which have made it a varied nutritious meal[1].

Walnut belongs to the family of *Juglandaceae* and has been widely used in traditional medicine throughout the world. The genus is called *Juglandaceae* because there are 5 – hydroxyl - 1, and 4-naphthoquinonein its leaf, fruit shell, wood and root[2]. Walnut oil, is extracted from English walnut (also known as the Persian walnut). This yellowish oil contains all lipid groups. The maximum percentage of which are triglycerides and sterile esters (96.9%).And only 3.1% of its lipids are polar lipids.73. 4%of polar lipids are sphingolipids and the remaining 26.6% are phospholipids. In walnut oil, however, these amounts are 2.3% and 0.8%, respectively[3,4]. Their major components are triglycerides, mono cyclic unsaturated fatty acids (mainly oleic acid) and polycyclic unsaturated fatty acids (linoleic and alpha-linolenic acid), which are present in high levels. The presence of other organic substances, such as phenols, tocopherols and phytosterols, has been alsoproved[3].

Walnut oil is also a good source of omega-3 fatty acids that are essential for human nutrition[4]. The major fatty acids found in walnut oil are linoleic, oleic acid and linoleic acid[5]. The preventive roles of monounsaturated fatty acids and polycyclic unsaturated fatty acids (MUFA and PUFA) in cardiovascular diseases have been identified.

It has been reported that the consumption of walnut (kernel and oil) lowers blood cholesterol levels[6,7]. Studies have shown that walnut oil has antioxidant properties and reduces coronary heart disease risks, inflammation, and is useful in the treatment of skin disease and high blood pressure[8-13]. Walnut kernels are used to reduce blood lipids, that is, to increase high density lipoprotein, and to reduce low density lipoprotein[14,15]. Walnut is also effective in the treatment of type 2 diabetes and enhancing cardiovascular flexibility [16].It has been reported that due to its high concentration of natural antioxidants, walnut can be consumed as a protection against certain types of cancer. It may also reduce the risk of cardiovascular diseases[17,18].

Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles[19], birds[20] and other vertebrates. In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females; however, small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid product. In men, testosterone has a key role in the development of male reproductive tissues like testis and prostate. In addition, it improves the secondary sexual characteristics such as increasing muscle mass, bone mass and hair growth[21]. Testosterone is also essential to health maintenance[22] and to prevent osteoporosis[23].

Testosterone is a major form of androgen derived from sterol C-19. Like all other steroid hormones, testosterone, is predominantly synthesized from cholesterol in the Leydig cells of the testes[24]. The testicular Leydig cells, are influenced by the central nervous system. The hypothalamus controls the pituitary gland, which results in the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in both sexes. However, females, mainly produce testosterone, and males mainly

produce estrogen[25]. In addition, based on the type of the receptor that is expressed in cells, different hormonal responses can be generated. The hypothalamus produces gonadotropin releasing hormones (G_nRH) which stimulate FSH and LH to enter blood circulation from the pituitary gland and then to spread to the Leydig cells in order to produce testosterone[26].

On the other hand, walnut can be considered as an effective drug for impotency because of its compounds such as: niacin, which plays an important role in the production of steroid hormones by the adrenal gland; arginine and aspartic acid which have stimulatory effects on G_nRH and LH; oleic acid which is a 5 - alpha reductase inhibitor; and bromine (Br) which increases estrogen in postmenopausal women.

Since very few studies had been conducted on the effects of walnut oil on the reproductive system[27-32], this survey was designed to study the effect of walnut oil on hormonal changes in the reproductive system of male rats, the results of which could be important in the area of pharmaceutical applications of walnut oil, especially in reproductive system.

MATERIALS AND METHODS

Animal

The present study was an experimental - laboratory research in which the control and the treated samples were compared. At first, a number of male and female rats within the weight range of 180-200gr and on the verge of puberty were purchased from Razi Vaccine & Serum Research Institute, Fars branch (Iran) and were kept in the animal center of Arsanjan Azad University. During the experiment, the animals were kept in standard conditions of temperature, light and moisture, and had free access to food and water. Before getting started, rats spent 4 days at adaptation condition to remove stress and to adapt to new conditions.

Measuring testosterone levels before and after puberty

Then rats were put together for mating. After the babies were born, male rats were isolated and 24 rats were randomly put into three groups of 8 rats as the pre-puberty group and the next 24 were similarly put into 3 groups of 8 rats as the post puberty group. For all the members of the pre- puberty group the test and gavage started at day 20 after birth and continued up to day 50 for 30 days. Then on the fiftieth day, blood samples were collected from rats. But in post puberty groups the test began on day 50 after birth, just when the rats reached puberty and it continued over a period of 30 days. On day 80 bloods sampling from theses rats was done.

Preparation of walnut oil and gavage

Walnuts were collected from Bavanat (Fars province of Iran) in late spring and a sample was kept in the herbarium of Medicinal Plants Research Center, Islamic Azad University of Arsanjan. After cleaning and drying in the shade, walnut kernels were ground into powder. Then, a certain amount of powdered walnut was kept in a solvent called n - hexane in the lab condition for 24 hours, then it was strained and the solution obtained was poured into the rotary device (RV10D, IKA England) at 40-50°C to let the solvent evaporate. To ensure that there was no moisture left it was kept in a desiccator device (GCD-051X, KIKO, Japan) which had a powerful vacuum pump, for another 24 hours. At the end a bright yellow oily substance with the concentration of 1.1363gr/mL suitable for gavage was obtained (Figure 1).Gavage was done once a day at 10 am for 30 days. At day 31, blood samples were taken from the animals. To do so, the animals were anesthetized by

chloroform and blood samples were taken from tail end. Then, blood serums were separated from blood samples; finally, by using a radioimmunoassay method (RIA) the serum testosterone levels were measured.

Statistical analysis

The results were analyzed by using SPSS version 11.5 and independent-sample T test.

RESULTS

Statistical analysis showed that the plasma testosterone level in pre-pubertal rats receiving walnut oil had significantly increased ($P<0.05$). Also in the groups receiving walnut oil post pubertally the testosterone levels significantly increased ($P <0.01$). Significant difference was observed between the post puberty groups and the pre- puberty ones ($P <0.001$). Table 1 represents the plasma levels of testosterone in the control, sham and experimental groups, pre and post puberty.

DISCUSSION

The results showed that testosterone levels in the experimental group receiving a dose of 20 g/ kg walnut oil had a significant increase both before and after puberty compared to the control group ($P <0.00$).According to the results of this research the increase in testosterone level indicates the positive effect of walnut oil on pituitary – testicular axis.

The hypothalamus - pituitary – testicular axis can be affected by various negative and positive feedbacks. Nitric oxide (NO) is one of the factors affecting this axis. High levels of Arginine in walnut can be converted to nitric oxide.

Nitric oxide increases the release of G_nRH, which in its turn increases gonadotropin secretion by activating neuron nitric oxide synthase enzyme in the pituitary gland[33,34].

Nitric oxide activates Guanylate cyclase enzyme that causes the release of cyclic guanosine monophosphate and eventually by raising G_nRH, LH and FSH, enhances sperm motility and induces erection in males[28].

Aspartic acid, which is one of the amino acids found in walnut, has a stimulatory effect on the secretion of LH and G_nRH. Experiments have shown that this amino acid regulates the synthesis of testosterone and LH through cyclic guanosine mono-phosphate (cGMP), and cyclic adenosine mono-phosphate (cAMP) as the second messengers in pituitary and testes, respectively[35]. Increased testosterone level in this experiment has had been the secondary consequence of increased G_nRH, particularly the LH.

Walnuts contains a large amount of polyunsaturated fatty acids such as linolenic acid, linoleic acid and oleic acid[36]. Studies have shown that in a large number of androgen-sensitive organs such as the prostate, testosterone via 5 alpha – reductase is converted to 5-alpha – dihydrotesto-sterone.

Unsaturated fatty acids are able to inhibit 5 alpha - reductase in cell cultures and cell-free systems. Isomers of cis-linolenic acid, linoleic acid and oleic acid have been shown to have great power in the inhibition of 5 alpha – reductase, thereby preventing the conversion of testosterone to dihydrotestosterone. In this way walnuts can prevent the reduction of plasma testosterone level[31]. However, it appears that the increase in testosterone levels with walnut oil was due to its direct effect on Leydig cells, as well as its interfere with the biosynthesis of testosterone which was probably done through stimulating the synthesis of prostaglandins series 2.

Walnut kernels contain alpha-linolenic acid, which can be converted to

arachidonic acid, as a precursor to make type 2 prostaglandins like E_2[37]. Arachidonic acid seems to play an important role in testicular steroidogenesis. As research indicates arachidonic acid increases cyclic adenylate cyclase, thus enhancing the rate of cholesterol side-chain breakage and stimulating the production of testosterone. So, these compounds mediate the testosterone production via messaging. Studies on a kind of fish showed that all E-series prostaglandins stimulated testosterone production in the testes.E_2wasmore powerful than E_1 and E_3[38].

Both n-3 and n-6 polyunsaturated fatty acids can affect reproductive processes through different mechanisms. They provide precursors for the synthesis of prostaglandins and ultimately regulate the expression patterns of key enzymes involved in the metabolism of steroids and prostaglandins[15].

CONCLUSIONS

The increase in testosterone concentration indicated the positive impact of walnut oil on the hypothalamic - pituitary - testicular axis. These positive effects could be done in several mechanisms some of which were mentioned in the discussion section above. According to this study, this effect was exerted both pre and post puberty. However, the post pubertal effects of walnut oil ingredients were much more than those of pre pubertal ones which could be caused by the completion of the hypothalamic–pituitary–gonadal axis (HPG axis).Yet, further study in this area is necessary.

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Conflict of interest

None declared.

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Table 1. Results of T test analysis of all pre-pubertal and post pubertal groups

P	Testosteroneng/ml	Group	
-	.84±.05	Control	*Pre-puberty*
NS	.88±.05	Sham	
P<0.05	1.34±.17	Experimental	
-	1.91±.14	Control	*Post puberty*
NS	2.07±.09	Sham	
P<0.01	3.70±.40	Experimental	

Values of P are presented in comparison to the sham group, represents no significant difference (NS) compared with control group.

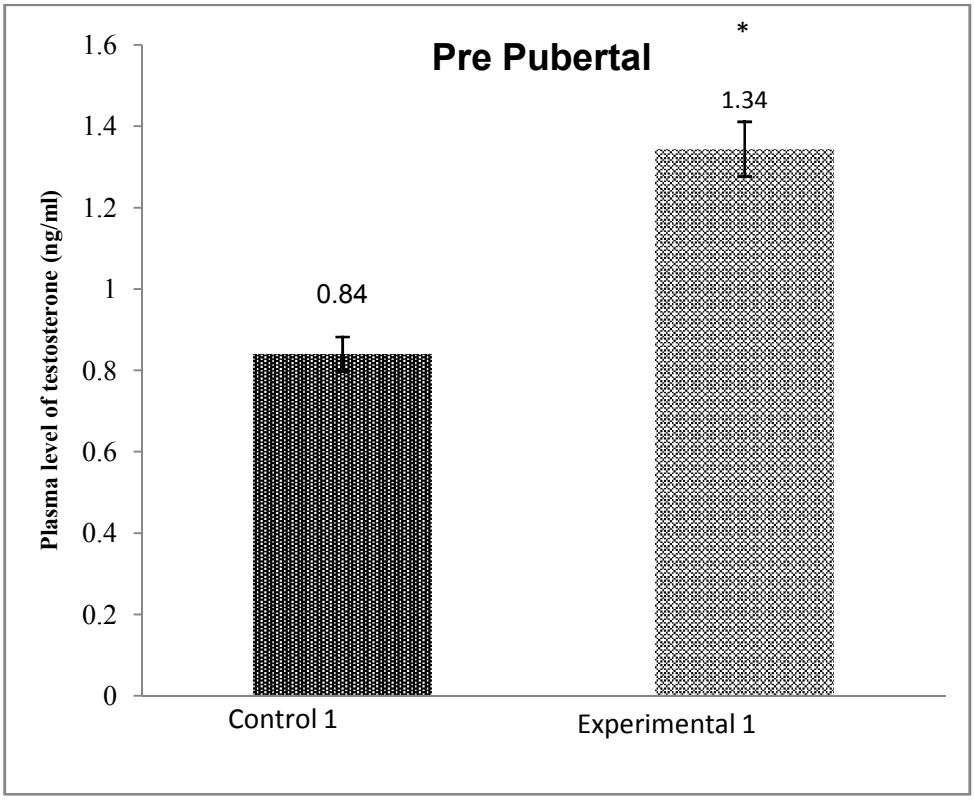

Figure 1. Compares the plasma testosterone levels between experimental and control groups in the pre-pubertal period. Each column represents the mean ± standard error (Mean ± SEM). Difference at P <0.05 was significant

* indicates significant difference with control group at P <0.05.

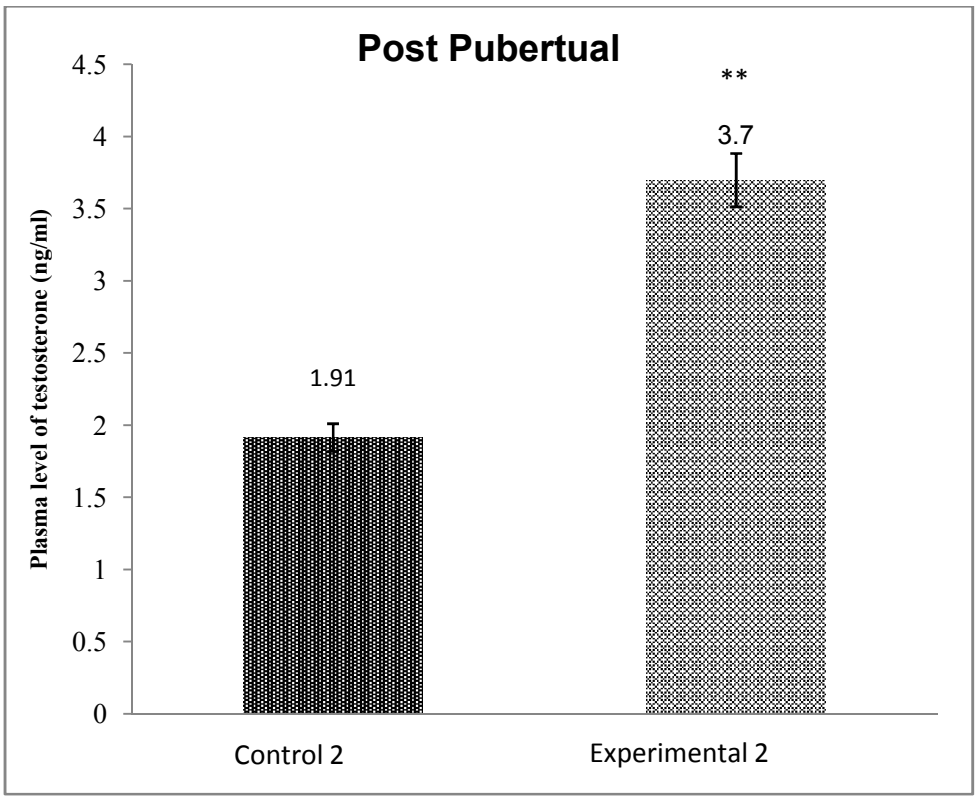

Figure 2. Compares the testosterone levels between experimental and control groups in post pubertal period .Each column represents the mean ± standard error (Mean ± SEM). Difference at P <0.01 was significant

**indicates significant difference with control group at P <0.01.

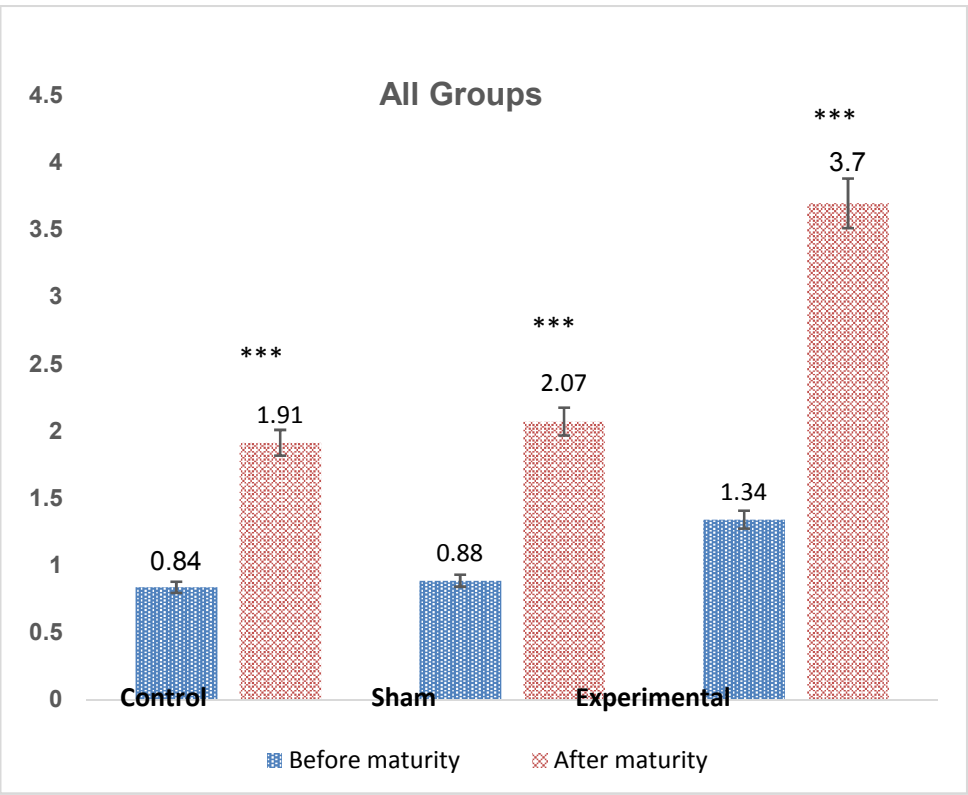

Figure 3. Compares the Testosterone levels among all groups of control, sham and experimental pre and post puberty

**indicates significant difference with pre and post-puberty groups at P <0.001.