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ORIGINAL ARTICLE

Effect of Prunus Amagdalis (Almonds) on testosterone in Atenolol-induced derangements in the hormonal profile of male Balb-c mice

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ABSTRACT

Background: Infertility is a very common health problem among middle-aged hypertensive individuals on beta-blocker therapy. With advancing research, it is observed that almonds can enhance the fertility index.
Aim: To find that how almonds (*prunus Amygdalus),* cardioselective beta blocker and combined preparation of both these can affect serum testosteronein mice.
Methods: This study was done in the Physiology Department, Shifa College of Medicine, Islamabad. Some part of this project was done inNational Institute of Health, Islamabad along with the Centre for Research in Experimental and Applied Medicine (CREAM) Laboratory, Army Medical College, Rawalpindi, and Shifa International Hospital, Islamabad. It was an Animal based experimental study. This study was conducted from December2014 to March 2015. 120 Balb-c mice were selected for our study according to the predefined setcriteria and four groups were made, each group consisting of 30 mice. Group A was the control group, atenolol was given to group B, almonds to group C, and atenolol plus almonds to group D. Serumtestosterone was measured after 3 months. Data was analyzed using SPSS version 17 and mean±standard deviation was determined. For difference, statistical significance was calculated after applying one way ANOVA. The p-value < 0.05 was considered significant.
Results: In group A serving as control mean testosterone was found to be 1.58±0.69IU/L. Mean serum testosterone in B-group (Atenolol) was 0.43±0.27 IU/L. In group C (Almonds) it was 3.77±2.64 IU/Lwhich was significantly increased. In group D (almond+Atenolol) serum testosterone level was1.82±1.00 IU/L that is elevated but non-significantly in comparison to the control group.
Conclusion: Almonds increase mean testosterone and atenolol lead to adecrease init. It is thus concluded that almondscan revert the changes induced by atenolol.
Keywords: Almonds, Testosterone, Atenolol, Hypertension, Infertility.

INTRODUCTION

Hypertension is one of the most common medical conditions in developing as well as developed nations and it is an important risk factor for different heart diseases leading to an increase in mortality[1]. Hypertension is labeled when systolic pressure rises more than 140mmHg and diastolic pressure is more than 90mmHg in adults[2]. The incidence is greater in males as compared to females. Several factors can affect the regulation of arterial pressure including urbanization, with its associated changes in lifestyle, weight, nutritional status and racial-ethnic differences[3]. Hypertension can lead to many pathophysiological changes such as atherosclerosis, inflammation, and endothelial dysfunction. Monitoring of blood pressure to reduce the risk of complications and lifestyle modifications along with drug therapy for better control of hypertension is the need of the hour[4].

Among all recommended drugs, beta-blockers are one of the good antihypertensive drug groups[5]. One of the common side effects of antihypertensive drugs in males is to decrease serum testosterone which is the leading cause of infertility[6]. Testosterone is an important hormone for male fertility. It is secreted by testicular interstitial cells of Leydig, stimulating differentiation of male phenotype and spermatogenesis in testes[7]. LH on binding with the receptor on the cell membrane of Leydig cell induces the breakdown of ATP (adenosine triphosphate) to form cyclic adenosine monophosphate (cAMP), which as a result catalyzes the synthesis of protein kinase A, essential fortestosterone production. Atenolol (beta blocker) causes a considerable reduction in testosterone release by decreasing the production of cAMP[5]. Male impotence can result from beta blocker-induced testosterone reduction and may lead to infertility in men.Atenolol may also affect the testicular weight and furthermore, it can affect the histomorphology of testes[8]. Deficiency of testosterone can

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In today's world, nuts have gained much attention among different dietary sources of micro and macronutrients due to their health benefits. One of the important nuts, almonds is considered beneficial for ages[10]. There are biochemical differences in different types of almonds and those are due to ecological conditions, genetic variation, and different repining harvest times[11].

A number of studies have reported almondsbeing used asan aphrodisiac, laxative, for curing headaches, enhancing mental retention power, and relief from insomnia[12]. Cholesterol-reducing effect of almonds is seen due to richness in monounsaturated fat (MUFA). It is very effective among hypercholesterolemic individuals. Though BMI modifies the effect of almonds on lipids profile but is always positive[13]. Many cardioprotective nutrients in almonds primarily target mechanisms for low-density lipoprotein Cholesterol (LDL-C) reduction[13].

Individuals with a good intake of nuts carry a reduced risk of different heart diseases[14]. The beneficial effects of Almonds on body weight are due to the fat and fiber composition of almonds which affects thedigestion of long-chain fatty acids, glucose regulation, oxidative stress, and inflammation[15,16]. Moreover, sperm motility, testosterone levels, and sperm count were increased without producing any lethal effect, establishing the protective role of almonds in the treatment of infertility and associated disorders[17].

The role of almonds in the prevention and treatment of antihypertensive drug-induced decline in testosterone has not been explored yet, so this study was planned to unravel this fact. This study will help to treat male infertility caused by drugs, and other causes as well.

MATERIAL AND METHODS

This animal experimental research was carried out at Physiology Department, Shifa College of Medicine, Islamabad in alliance with the experimental Laboratory of its affiliated hospital, Centre for Research in Experimental and Applied Medicine (CREAM) laboratory, Army Medical College Rawalpindi and National Institute

of Health, Islamabad on mice. Study was conducted after being approved by the ethical review board of the institute. Mice of breed BALB-c were obtained from the animal house of the National Institute of Health, Islamabad. Non-probability convenience sampling was used to divide 120 animals into 4 equal groups. According to inclusion criteria, adult BALB-c mice 42-56 days in age and having 25±5 grams body weight and normal gonads were part of our study, the animals not fulfilling this criterion (like diseased ones or underweight or under age) were excluded from the experiment.

Twelve hours of light and twelve hours of the dark cycle, already followed in the animal house of NIH Islamabad, were maintained for the mice according to standards. Clean water bottles were fitted over their enclosure.

The kernels of almonds were bought from the regional market which was further tested and certified by the National Agricultural Research Centre (NARC), Islamabad. A fine mixture of almonds was obtained by grinding them. An aqueous solution of ground kernels was made to be used as an extract[17]. Atenolol in the pack of 5-gram Cat No: A-7655 was purchased from Sigma USA. MP Bio USA was the source of our solventDMSOCat No:191418 in a pack of 500 ml.Atenolol was dissolved in DMSO for administering it orally to mice in this experiment. Dietary supply and water for mice were given ad libitum and they were kept at74±2 ^{o}F temperature.

The Control group in this experiment was Group A. Thisgroup was provided with 1ml water and 1ml dimethyl sulphoxide (DMSO) per oral once a day for 90 days through a gavage tube. Atenolol (cardioselective beta blocker) was given toGroup B and at a dosage of 18 mg per kg body weight per ml of DMSO orallythrough a gavage tube, one time a day for 90 days.[18]Group C was administered100mg/kg extract of almonds per oral through a gavage tube, one time a day for 90 days[17].Group D was provided with both substances in doses, atenolol 18 mg/kg body weight/ml of DMSO, and almond extract100mg/kg per oral through a gavage needle, once a day for 90 days[17,18]. One mouse from a total of 120 died in the mid of the study, this mouse was from the B Group(atenolol); while the rest of the 119 mice remained safe and healthy in the total study period.

Blood sampling from heartof mice was done. 1.5 ml of bloodfrom every animal was drawn at the end of 90 days study period. The blood was run at a rate of three thousand revolutions per minute through a centrifuge machine for 10 minutes.

Serum was obtained and by using micropipettes was kept and stored in Eppendorf tubes at -94^{0}F to -112^{0}F temperature in the CREAM lab, Army Medical College for detection ofserum testosterone.

Estimation of serum testosterone was done running a Mouse Testosterone kit with catalog Number5827071 by Cayman's Chemicals U.S.A. The technique employed in this kit isquantitative sandwich enzyme immunoassay.

Version 17 of SPSS was used for analyzing data. Meanwith ± standard deviation was determined for serum testosterone levels. We applied one-way ANOVA todetermine the statistical significance of differences in the results and less than 0.05 p-value was taken as significant.

RESULTS

Four equal groups of animals were made out of a total of one hundred and twenty.One hundred and nineteen mice remained healthy and active till the end of the experiment but one mouse which belonged to the B group (Atenolol) could not survive and expired duringthe second month of the experiment. After 90 days,mean serum testosterone level of all mice was determined which is presented in table I. Group B(Atenolol) showed a significant reduction in testosterone levels in comparison to control group A. On the other side Group C (Almonds) showed a rise in mean serum testosterone level significantly when compared to the control group. The mean level of serum testosterone wasincreased in group D but non-significantly in comparison to the control group. For further analysis, ANOVA was applied followed by posthoc Tukey's test to compare all study animals group wise with each other and this is shown in table II.

In analysis of group B with group C and group D, a significant rise in testosterone level was observed in both C and D groups. Significant elevation inserum testosterone level ofC group was found when it was compared with the D group.

Analysis between (Almond) group C and combination (Atenolol+Almond) group D showed significantrise in testosterone level in group C (Almond).

Table I: ±Mean values ±SD of Serum Testosterone level(nm/l)

Study Groups	Testosterone(IU/L)	p-value
Group A(Control)	1.58 ± 0.69	0.000*
Group B(Atenolol)	0.43±0.27	
Group C (Almonds)	3.77 ± 2.64	
Group D (Atenolol+Almonds)	1.82 ± 1.00	

*Significance (p<0.05) at confidence interval of 95%. One way ANOVA was applied.

All values are expressed as mean ± standard deviation.

Table II: Serum testosterone level among all groups compared by applying ANOVA and followed by post-hoc Tuckey's test

Comparison of Groups	p-value
Control group (group A)	
Atenolol group-B	0.020 *
Almond group-C	0.000*
Atenolol+Almonds group-D	0.930
Atenolol group (group B)	
Almond group-C	0.000*
Atenolol+Almonds group-D	0.003*
Almond group (Group D)	
Atenolol+Almonds group-D	0.000*

*Significance (p<0.05) at confidence interval of 95%.

DISCUSSION

In this study, atenolol was administered to a group of 30 male Balb-c mice. A significant reduction was found in the level of serum testosterone in this group. Observations in our research are consistent with the findings of an experiment conducted by El-Sayed et al, who observed the effects of atenolol, metoprolol, and propranolol on male rat fertility. All rats treated with these drugs showed a decrease in the level of testosterone hormone significantly.[18]

Khan et al conducted a study on isolated Leydig cell culture on Wistarmalerats. According to this study atenolol, in different concentrations led to a significant decrease in testosterone release from Leydig cells when compared to the baseline release of the hormone in different doses. They also concluded that atenolol reduced testosterone release by Leydig cells which were stimulated by luteinizing hormonemore significantly as compared to the effects of atenolol onLeydig cells which were not stimulated by luteinizing hormone. The mechanism of action of atenolol as manifested by their study suggested indirectly that atenolol inhibits testosterone release by reducing the production of cAMP. This result showing a decrease in testosterone induced by atenolol on cultured testicular Leydigcells was consistent with the results of our study[19].

The effect of a herbal administration that included almonds along with other constituents on male Wistar rats was analyzed in a study conducted by Gopumadhavan et al. On the 5th day, 60 minutes after the drug administration mounting latency, mounting frequency, ejaculation latencyand ejaculation frequency were observed. Blood sampling was done from retro-orbital plexus for the measurement of serum testosterone. All parameters ofthe present study in the group treated by herbal formulation including almonds were significantly improved. Moreover, a rise in sperm count and serum testosterone levels was observed. This effect of an increase in thenumber of sperms and mean testosterone level

is similar to the increase in both parameters observed after feeding the mice with almonds in our study[20].

Kalgaonkar et al demonstrated the effect of walnuts and almonds on lipid profile and free androgen index in thirty-one PCOS (Polycystic Ovarian Syndrome) patients. These patientsrandomly received either almonds or walnuts every day for 6 weeks. According to the results, their weight remained stable and there was a decrease in unhealthy and bad cholesterol levels as well as a decrease in serum testosterone level by reducing free androgen index. The effect of almonds on testosterone shown in this study is different from the results of the present study. Thisobservation may be because of the difference in dosage and length of treatment, and most importantly they conducted their study on female humans, whilethe present study is on male mice[21].

In this study, group D received both substances atenolol and almond extract to find whether almond extract can neutralize the fluctuations produced by adverse effects of atenolol inreproductive physiology. According to findings of present study extract of almond extractwas found to be capable ofreverting changes in the reproductive system and it reversed derangements in serum testosterone levels which were triggered by use of Atenolol. The values obtained from this group were not significantly different from the control group. The mechanism of causing these derangements is the decrease in the production of cAMP which leads to a decrease in the testosterone synthesis.[19] Almond extract was able to revert these changes probably because of the occurrence of severalvital ingredients in almonds like alpha-tocopheroland monounsaturated fatty acids.[22] These elements found in almonds may not actually revert the effects of atenolol, but throughother different mechanisms,can improve the general fertility parameters, and derangements produced by atenolol. Many studies show that alpha-tocopherol, through its role against oxidative stress, exerts a good impact onthe functioning of testes and quality of semen.Semen quality and fertility are both affected by oxidative stress. In addition, high levels of free radicals are closely related to infertility problems and affectnatural conception or evenfailure of assisted pregnancy. Zinc, selenium, and arginine also improve some semen parameters and all these are present in *Prunusamygdalis*so their role in the improvement of changes caused by atenolol cannot be ignored[23]. Testosterone is the main male reproductive hormone required for the structural and functional integrity of testes and male fertility, so we can use the agents showing positive effects on its production. If these agents are consumed in the form of delicious nuts then there will be more compliance for their intake and ultimate benefit.[22]

CONCLUSION

Testosterone production was increased by the use of prunusamygdalis independently and Atenolol-induced decline was prevented as well. Therefore, this study proved that the use of almond extract is beneficial in the treatment of testosterone deficient infertility in males.

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REFERENCES

1. Koivistoinen T, Lyytikäinen L, Aatola H, Luukkaala T, Juonala M, Viikari J, *et al.*Pulse Wave Velocity Predicts the Progression of Blood Pressure and Development of Hypertension in Young Adults. Hypertension 2018;71: 451–6
2. Lee CJ, Park WJ, Suh JW, Choi KE, Jeon DW, Lim SW, *et al.* Relationship between health-related quality of life and blood pressure control in patients with uncontrolled hypertension. J Am CollCardiol 2018; 71: 1304-34.
3. Choi HM, Kim HC, Kang DR. Sex differences in hypertension prevalence and control: Analysis of the 2010-2014 Korea National Health and Nutrition Examination Survey. PLoS One 2017;12; 2-12.
4. Antonioa C, Jaramillo L, Patriciob C, Costasd T, Zanchetti, Albertoe. Best antihypertensive strategies to improve blood pressure control in Latin America: position of the Latin American Society of Hypertension. J Hypertens 2018; 36: 208–20.
5. Oyedeji KO, Robert E, Arubi P, Dare A. Effect of Atenolol (Beta Blocker) on Reproductive Parameters in Male Wistar Rats. Int J Pharm Sci Res 2018: 10: 497-500.
6. PJ Snyder, S Bhasin, GR Cunningham, Matsumoto AM, Shields AJS, Cauley JA, *et al.*. Lessons from the testosterone trials. Endocr Rev 2018: 39; 369-386.
7. Wangab Y, Huangenbc S, Z Wang, F Chen, P Chen Zhahoa X, HanLina *et al.* Long-term maintenance of luteinizing hormone-responsive testosterone formation by primary rat Leydig cells in vitro. Mol Cell Endocrinol 2018; 476: 48-56.
8. Ali L, Naveed AK, Minhas LA, Tahir M. Effects of the Propranolol on Morphology of Adult Rats Testis. Int J Pathol 2007; 5: 54-7.
9. Hackney AC, Aggon E. Chronic Low Testosterone Levels in Endurance Trained Men: The Exercise- Hypogonadal Male Condition. J BiochemPhysiol 2018; 1: 103-4.
10. Jung H, Chen CYO, Blumberg JB, Kwak HK. The effect of almonds on vitamin E status and cardiovascular risk factors in Korean adults: a randomized clinical trial. Eur J Nutr 2018; 57: 269–79.
11. Oliveira I, Meyer AS, Afonso S, Ribeiro C, Goncalves B. Morphological, mechanical and antioxidant properties of Portuguese almond cultivars. J. Food Sci Technol. 2018; 55:467–78.
12. N Gorji R, Moeini Z, Memariani. Almond, hazelnut and walnut, three nuts for neuroprotection in Alzheimer's disease: A neuropharmacological review of their bioactive constituents. Pharmacol Res 2018; 129: 115-27.
13. Zibaeenezhad MJ, Ostovan P, Mosavat SH, Zamirian M, Attar A. Almond oil for patients with hyperlipidemia: A randomized open-label controlled clinical trial. Complement Ther Med 2019; 42: 33-6.
14. Tahir FN , Shah SIA , Danyal M , Quresh JA. Effect of Almond Consumption on Serum Lipid Profile in Dyslipidemic Adults.Pak J Med Health Sci 2018; 12: 799-801.
15. Liu Y, Hwang HJ, Kim HS, Park H. Time and Intervention Effects of Daily Almond Intake on the Changes of Lipid Profile and Body Composition Among Free-Living Healthy Adults. J Med Food 2018; 21: 340–7.
16. Xiao Y, Xia J, Ke Y, Cheng J, Yuan J, Wu S, *et al.* Effects of nut consumption on selected inflammatory markers: a systematic review and meta-analysis of randomized controlled trials. Nutrition 2018; 54: 129-43.
17. Qureshi S, Shah AH, Tariq M, AgeelAM. Studies on herbal aphrodisiacs used in Arab system of medicine. Am J Chin Med 1989; 17:57-63.
18. El-Sayed MG, el-Sayed MT, ElazabAbd el S, Hafeiz MH, el-Komy AA, Hassan E. Effects of some beta-adrenergic blockers on male fertility parameters in rats. DtschTierarztlWochenschr 1998; 105:10-2.
19. Khan UA, Aslam M, Saeed SA . Effect of beta adrenergic antagonist on the production of testosterone by rat's Leydig cells. J Ayub Med Coll 2004; 16: 26-8.
20. Gopumadhavan, S., Mohamed Rafiq, Venkataranganna, M.V., Kala Suhas Kulkarni, and Mitra, S.K. Assessment of 'Tentex royal' for sexual activity in an experimental modelInt J ClinPract 2003:10 :23-6.
21. Kalgaonkar S, Almario RU, Gurusinghe D, Garamendi EM, Buchan W, Kim K, et al. Differential effects of walnuts vs almonds on improving metabolic and endocrine parameters in PCOS. Eur J ClinNutr 2011; 65: 386-93.
22. Sohrabvand F, Mahroozade S, Bioos S, Nazari SM, Dabaghian FH. Improvement in Sperm Parameters With Traditional Iranian Remedy: A Case Report. Evid Based Complement Alternat Med. Journal 2017; 22: 223-6.
23. Salunkhe S. Efficacy of sweet almond oil in corticosteroid modified Tinea (Tinea Incognito). World J Pharm Res 2018; 7; 428-38.