ORIGINAL ARTICLE

EFFECT OF ALMONDS AND ATENOLOL ON TESTICULAR WEIGHT IN MALE BALB-C MICE

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Background: *Prunus amygdalis* (almond) is focus of attention these days because of many benefits. It also acts as hypocholesterolemic agent so decreases hypertension risk factors. Complications of hypertension are increasing constantly. Beta blockers are frequently used antihypertensive agents. Atenolol (a β-blocker) also shows some side effects, it decreases testicular weight and almonds increase it. Study was designed to observe the effects of *Prunus amygdalis* and Atenolol on testicular weight to reveal that *Prunus amygdalis* can revert Atenolol's effect or not. **Methods:** One hundred and twenty mice fulfilling inclusion criteria were divided into 4 equal groups. Group A served as control, group B was given Atenolol, group C was given almonds and group D was given both Atenolol and almonds. After 3 months, testes were dissected and weighed. Data was analyzed on SPSS-17, Mean±SD was calculated. One-way ANOVA was applied and $p \leq 0.05$ was considered significant. **Results:** The mean testicular weight in Group A was 0.127±0.01 gms, in Group B it was 0.113±0.01 gms, in Group C and Group D testicular weight was 0.136±0.01 gms and 0.120±0.01 gms respectively. When Group A was compared with Group B it was significantly reduced and no significant difference was found in Group C and Group D in comparison to Group A. **Conclusion:** Administration of *Prunus amygdalis* does not significantly affect testicular weight but Atenolol causes a significant decrease in it that can be reversed by *Prunus amygdalis*.

Keywords: Almonds, Cardioselective Beta Blocker, Atenolol, Testicular Weight

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INTRODUCTION

As emerging research reveals different facts about healthy and nutritious snacks like nuts, so these are focus of attention these days.[1] In late 20th century nuts were considered as harm to health and it was advised to limit their use.[2] Recently many studies are being conducted to understand more about the advantageous and risky effects of nuts on Health.[3] *Prunus amygdalis* commonly known as almond, is a center of attention these days.[4] *Prunus amygdalis* has one of its own kind of composition containing monounsaturated fat, fibre, α-tocopherol, minerals such as magnesium and copper, and phytonutrients etc.[5] Its unique lipid and fiber combination contributes to the hypocholesterolemic effect of almond consumption.[6] These all effects lead to a decrease in adaptable cardiovascular and diabetes risk factors such as body mass index and glucose resistance.[7] It also has beneficial role in inflammation and oxidative stress.[8] Almond oil was also a part of research for its valuable effects on striae gravidarum and found to be helpful in this regard.[9] It also has other good effects such as sclerosing agent in treatment of rectal prolapse.[10] *Prunus amygdalis* has so many other different properties such as antioxidant and immunostimulant.[11] Almonds also acts as laxative agent.[12] Furthermore it is fertility enhancer and memory restorative agent.[13]

According to World Health Organization the incidence of hypertension along with its awful complications is on a rise.[14] To ensure quality life and to keep one free from complications of hypertension, its effective and timely management is mandatory. A good management involves life style modifications and use of various drugs.[15] Beta antagonists are one of the effectively and commonly used drugs, amongst which the beta-1 adrenoceptor blocking agents like Atenolol are preferred.[16] Like all other drugs, along with its beneficial pharmacological effects it also exerts some side effects. It is not good for male reproductive system and leads to a decrease in testicular weight affecting male fertility.[17] The present study was designed to observe the effects of *Prunus amygdalis* and Atenolol on testicular weight.

MATERIAL AND METHODS

It was randomized control trial conducted on male BALB-c mice from December 2015 to July 2016. After approval from the Ethical Committee the study was carried out in Physiology Department, Shifa College of Medicine, Islamabad in collaboration with Laboratory of Shifa International Hospital Islamabad, National Institute of Health, Islamabad and Centre for Research in Experimental and Applied Medicine, Army Medical College, Rawalpindi. BALB-c mice were obtained from the animal house of National Institute of Health,

Islamabad. A sample size of 120 was calculated using WHO sample size calculator. The mice were divided into 4 equal groups through non-probability convenience sampling. Adult 6–8 weeks old, male, BALB-c mice having body weight 25±5 grams with both testes normal were included in the study. Mice having any disease or with abnormal testes examined by veterinarian were excluded from study. Mice were kept in 12-hour light/dark cycle. The seeds of *Prunus amygdalis* (sweet almonds) were purchased from local market. Almonds were ground to get fine mixture, which was then dissolved in water and this material served as extract.[18] Atenolol used in the experiment was taken from Sigma USA in a packing of 5 gram Cat No: A-7655. Dimethyl sulphoxide (DMSO) was used from MP Bio USA Cat No: 191418 in packing of 500 ml. DMSO was used as solvent for Atenolol in this study. The mice were supplied with food and water ad libitum. The temperature for mice was kept at 23±2 °C.

Group A (n=30) was control group. These mice were given 1 cc water and 1 cc DMSO orally once daily for 3 months with gavage needle. Group B (n=30) was given Atenolol in dose of 18 mg/Kg body weight/ml of DMSO orally with gavage needle, once daily for 3 months.[19] Group C (n=30) was given extract of almonds in a dose of 100 mg/Kg orally with gavage needle, once daily for 3 months.[18] Group D (n=30) was given Atenolol in a dose of 18 mg/Kg body weight/ml of DMSO, and almond extract in a dose of 100 mg/Kg orally with gavage needle, once daily for 3 months.[18,19]

Out of 120 mice, 119 mice remained alive and healthy throughout the study but 1 mouse from group B which was Atenolol treated group died during the study. After completion of 3 months, mice were anesthetized with chloroform and then testes were dissected out and weighed by placing on a digital balance in laboratory of animal house of NIH.

Data were analyzed using SPSS-17. Mean±SD was calculated for testicular weight. The statistical significance of differences across the results was determined by applying one-way ANOVA followed by post-hoc test, and $p \leq 0.05$ was considered significant.

RESULTS

Mean testicular weight in all groups at the end of three months is presented in Table-1. In group B the mean testicular weight was significantly lower than control group (A). In group C the mean testicular weight was more than that of control group but difference was not statistically significant. Group D had mean testicular weight less than that of control group but difference was not significant again.

Comparison of all these values with control group after applying one way (ANOVA) post hoc Tukey's *t*-test is shown in Table- 2.

Table-1: Testicular weight (gms) among all groups (Mean±SD)

Groups	Testicular Weight (gms)	*p*
Group A (Control) (n=30)	0.127±0.01	<0.001*
Group B (Atenolol) (n=30)	0.113± 0.01	
Group C (Almonds) (n=30)	0.136±0.01	
Group D (Atenolol+Almonds) (n=30)	0.120±0.01	

*Significant

Table-2: Comparison of testicular weight (gms) among groups analysis of variance (ANOVA) followed by post-hoc Tukey's *t*-test

Group wise comparison		*p*
Group A (Control) (n=30)	Group B (Atenolol)	0.001*
	Group C(Almonds)	0.065
	Group D (Atenolol+Almonds)	0.159
Group B (Atenolol) (n=30)	Group C (Almonds)	<0.001*
	Group D (Atenolol+Almonds)	0.265
Group C (Almonds) (n=30)	Group D (Atenolol+Almonds)	<0.001*

*Significant

DISCUSSION

Atenolol, a cardioselective beta blocker is widely used as an antihypertensive agent but it causes various side effects including male infertility.

Donta *et al*[20] conducted a study on effects of beta blocker (propranolol) on body weight in 144 Wistar rats of both sexes from weaning to 17 weeks of age. Drug was administered to 77 of these rats (chosen at random). Body weights were monitored every third day. During their 17th week of age, all the rats were weighed for the last time and then sacrificed. These organs were dissected and weighed: brain, thymus, lungs, heart, liver, kidneys, adrenals, testes and ovaries. The weight of testes did not decrease in that study after administration of beta blocker while in the present study beta blocker reduced testicular weight. This difference may be because of different species used in both studies. Duration of treatment, and the beta blocker used in the two studies was also different.

Naveed *et al*[21] conducted a study to observe some effects of β-adrenergic receptors antagonist on testicular weight. They carried out this study by using fifty-six adult Sprague-Dawley rats, divided them in 4 groups and along with other parameters observed the dissected out testes and weighed them. There was no significant difference in testicular weight of control and treated groups. This effect does not match with the results of present study in response to beta blockade. This difference in result may be because of the difference in compound used. The cause of this difference might also be the difference in species or duration of treatment.

Qureshi *et al*[18] observed the effects of *Prunus amygdalis* on sperm morphology (percentage of abnormal sperms) and testicular weight. They concluded that administration of almonds did not cause

any significant difference in both parameters when compared with control. This finding is consistent with the findings of our study.

Mesbah *et al*[22] took five groups of Sprague-Dawley adult male rats (n=72). Two groups were medicated with intramuscular injection of nandrolone decanoate and other two groups with same doses of sweet almond and olive oils, respectively, for 14 weeks. One week after the last injection, rats were sacrificed and the weights of the body, testes and epididymis were assessed. The weights of testes and epididymis were significantly decreased in nandrolone decanoate treated groups while in the sweet almond and olive oils treated groups and in control group there was no significant differences. The results of that study are not consistent with present study. This difference might be because of the reason that we used full almond while Mesbah *et al* used only its oil. In present study group D was given both Atenolol and *Prunus amygdalis* to see whether *Prunus amygdalis* is capable of neutralizing the changes produced by adverse effects of Atenolol on reproductive system. *Prunus amygdalis* was found beneficial for reproductive system and it reversed decrease in testicular weight caused by Atenolol. The mechanism how Atenolol causes these derangements is the decreased production of cAMP leading to decrease in production of testosterone, hence may be a reason for decreasing testicular weight.[23] The probable mechanism by which almonds were able to revert these changes may be because of presence of various important ingredients in almonds like α-tocopherol, mono-unsaturated fatty acids and other minerals which were found to be present in almonds by Maguire *et al*.[24] Another study by Shalaby *et al*[25] showed that alpha tocopherol through its action against oxidative stress, also improves other fertility parameters such as the quality of semen. A certain level of free radicals is essential for normal sperm function, but an excessive level of free radicals can cause harmful effect on testicular function and subsequent fertilization. Oxidative stress builds up when there is an imbalance between generation of free radicals and scavenging capacity of anti-oxidants in reproductive tract. In addition, high levels of free radicals have been associated with lack of or poor fertility product after natural conception or assisted reproduction. Administration of antioxidants improves spermatozoid production along with some other fertility parameters.

CONCLUSION

Administration of *Prunus amygdalis* does not significantly affect testicular weight but Atenolol causes a significant decrease in testicular weight that can be reverted by the use of *Prunus amygdalis* and it can be useful in the treatment of Atenolol induced male infertility.

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REFERENCES

1. Bitok E, Sabate J. Nuts and Cardiovascular Disease. Prog Cardiovasc Dis 2018;61:33–7.
2. Rusu MR, Simedrea R, Gheldiu AM, Mocan A, Vlase L, Popa DS, *et al* Benefits of tree nut consumption on aging and age-related diseases: Mechanisms of actions. Trends Food Sci Tech 2019;88:104–20.
3. Zadeh NA, Ahmadi K. The impact of several plant extracts and essential oils on pistachio psylla (*Agonoscena pistaciae*) and its natural enemy. J Nuts 2018;9:159–68.
4. Bowen J, Luscombe-Marsh ND, Stonehouse W, Tran C, Rogers GB, Johnson N, *et al.* Effects of almond consumption on metabolic function and liver fat in overweight and obese adults with elevated fasting blood glucose: A randomised controlled trial. Clin Nutr ESPEN 2019;30:10–8.
5. Holscher HD, Taylor AM, Swanson KS, Novotny JA, Baer DJ. Almond consumption and processing affects the composition of the gastrointestinal microbiota of healthy adult men and women: a randomized controlled trial. Nutrients 2018;10:126–36.
6. Kalita S, Khandelwal S, Madan J, Pandya H, Sesikeran B, Krishnaswamy K. Almonds and cardiovascular health: a review. Nutrients 2018;10:468–76.
7. Pedersen S, Sniehotta FF, Sainsbury K, Evans EH, Marques MM, Stubbs RJ, *et al.* The complexity of self-regulating food intake in weight loss maintenance. A qualitative study among short- and long-term weight loss maintainers. Soc Sci Med 2018;208:18–24.
8. Batool Z, Tabassum S, Siddiqui RA, Haider S. Dietary Supplementation of almond prevents oxidative stress by advocating antioxidants and attenuates impaired aversive memory in male rats. Plant Foods Hum Nutr 2018;73:7–12.
9. Hajhashemi M, Rafieian M, Rouhi Boroujeni HA, Miraj S, Memarian S, Keivani A, *et al.* The effect of *Aloe vera* gel and sweet almond oil on striae gravidarum in nulliparous women. J Matern Fetal Neonatal Med 2018;31:1703−8.
10. Dolejs SC, Sheplock J, Vandewalle RJ, Landman MP, Rescorla FJ. Sclerotherapy for the management of rectal prolapse in children. J Pediatr Surg 2018;53:73−6.
11. 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;57:2069–79.
12. Chen CYO, Milbury PE, Blumberg JB. Polyphenols in almond skins after blanching modulate plasma biomarkers of oxidative stress in healthy humans. Antioxidants (Basel) 2019;8:95−9.
13. Bhardwaj R, Dod H, Sandhu MS, Bedi R, Dod S, Konat G, *et al*. Acute effects of diets rich in almonds and walnuts on endothelial function. Indian Heart J 2018;70:497–501.
14. Kumar R, Kumar H, Kadiyan J. Prospective study to estimate the prevalence and incidence of hypertension in adults below and above 35 years of age. World J Pharm Res 2018;7:2431−3.
15. Efremova E, Shutov A, Suvorova S, Tolstyga M, Vydrina S, Troshina I, *et al.* Adherence to lifestyle therapy and drug therapy in patients with arterial hypertension and comorbidity. J Hypertens 2019;37:e251–6.
16. Leavitt K, Običan S, Yankowitz J. Treatment and prevention of hypertensive disorders during pregnancy. Clin Perinatol 2019;46;173–85.

17. Sabidó M, Thilo H, Guido G. Long-term effectiveness of bisoprolol in patients with angina: A real-world evidence study. Pharmacol Res 2019;139;106–12.
18. Qureshi S, Shah AH, Tariq M, Ageel AM. Studies on herbal aphrodisiacs used in Arab system of medicine. Am J Chin Med 1989;17:57–63.
19. Weick RF. Acute effects of adrenergic receptor blocking drugs and neuroleptic agents on pulsatile discharges of luteinizing hormone in the ovariectemized rat. Neuroendocrinology 1978;26:108–17.
20. Donta I, Karayannacos PE, Boudoulas H, Kostakis A, Sechas M, Varonos D, *et al*. Effect of beta-adrenergic blockade on physiologic growth in the Wistar rat. Res Commun Chem Pathol Pharmacol 1982;37:147–50.
21. Naveed AK, Ali L, Minhas LA, Tahir M. Effects of propranolol on testosterone in adult rats. Int J Pathol 2007;5:54–7.
22. Mesbah SF, Shokri S, Karbalay-Doust S, Mirkhani H. The effect of nandrolone decanoate on the body, testis and epididymis weight and semen parameters in adult male rats. Iran J Med Sci 2007;32:93–9.
23. 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 Abbottabad 2004;16(4):26–8.
24. Maguire LS, Osullivan SM, Galvin K, O'Connor TP, Obrien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. Int J Food Sci Nutr 2004;55:171–8.
25. Shalaby MA, el-Zorba HY, Kamel GM. Effect of alpha-tocopherol and simvastatin on male fertility in hypercholesterolemic rats. Pharmacol Res 2004;50:137–42.

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