ORIGINAL ARTICLE

Effect of Almonds and Atenolol on Sperm Morphology in male Balb-C Mice

IRUM REHMAN[1], AFTAB AHMAD[2], NAUREEN WASEEM[3], FAIZANIA SHABBIR[4], MARIA GILL[5], AMINA RASUL[6], MEHWISH ASHFAQ[6]

[1]*Associate Professor/Head of Department of Physiology, Margalla Institute of Health Sciences, Rawalpindi.*
[2]*Associate Professor, Department of Anatomy, M. Islam Medical College, Gujranwala.*
[3]*Associate Professor, Department of Anatomy, Islam Medical College, Sialkot*
[4]*Associate Professor, Department of Physiology, Gujranwala Medical College, Gujranwala*
[5]*Associate Professor, Department of Physiology, CMH Institute of Medical Sciences, Multan*
[6]*Associate Professor, Department of Physiology, Watim Medical College, Rawalpindi.*
[7]*Assistant Professor, Department of Physiology, HITEC- Institute of Medical Sciences, Taxilla.*
Correspondence to Dr. Irum Rehman, Email: junaidirum@gmail.com. Cell: 03217826878.

ABSTRACT

Aim: To evaluate potential effects of Almonds (Prunus Amygdalis) on sperm morphology as such and after administration of Atenolol.

Methods: This laboratory-based randomized control trial was conducted out in the Physiology Department, Shifa College of Medicine, Islamabad. The Laboratory of Shifa International Hospital, Islamabad and CREAM lab of Army Medical College, Rawalpindi collaborated in this study from December, 2015 to July, 2016 on 120 Balb C mice. Animals were divided into four groups (n=30). Animals in group A(Control) were given 1 cc water and 1cc DMSO(Atenolol solvent). Group B mice were givenAtenolol 18 mg/Kg body weight/ml of DMSO, group C mice were given almonds extract 100mg/Kg. Group D mice (n=30) were given Atenolol 18 mg/Kg body weight/ml of DMSO, andalmond extract 100 mg/Kg, these agents were administered orally once daily for 3 months. After

Results: Percentage of abnormal sperms was 1.580% ± 0.67% in-group A(control). Therewas significant increase in-group B (Atenolol) to 13.762% ± 5.31%. In-group C(Almond) it decreased to 1.242% ± 0.76% and in-group D(Atenolol+Almond) it was increased to 1.615 ± 0.96% as compared to group A but not significantly.

Conclusion: Prunus Amygdalis not only has beneficial effects on sperm morphology innormal mice but can also reverse atenolol-induced abnormalities.

Keywords: Atenolol, Almonds, Prunus Amygdalis, Sperm Morphology, Balb C mice.

INTRODUCTION

Diet has an essential role in the health maintenance along with the disease prevention. Adding nuts to our lifestyle has become a focus of attention these days. Earlier in the 20th century, it was advised to limit their use due to its harmful effects. There have been many researches recently to figure out the role of nuts on human health. Prunus amygdalis is one of such a nut family, which has gained a lot of attention these days. “Prunusamyedalisare” rich in minerals such as copper, magnesium and potassium, arginine, α–tocopherol, monounsaturated fat, fibre, and phytonutrients etc. Almond has a hypocholesterolemic effect due to its lipid and fiber combination. All these effects lead to a decrease in diabetes and cardiovascular risk factors such as glucose resistance and body mass index. It has also been found beneficial role in oxidative stress and inflammation. Researches have proven that they are valuable effects of almond oil on striae gravidarum. In patients with rectal prolapse, it can be used as a sclerosing agent. Prunus amygdalis has some antioxidant and immunostimulant and laxative properties too. Furthermore, almonds are memory restorative agents and fertility enhancers as they are rich in arginine. Reproductive performance and male sexual behavior depend on the circulating levels of testosterone in the blood, which are altered by the use of almonds. Almonds are important for testosterone production as they are great sources of calcium, magnesium and potassium. According to World Health Organization, there is a high incidence of hypertension and its deleterious complications are on a rise. Hypertension needs to be effective and timely managed to ensure quality life and prevent complications. Good management involves lifestyle modifications along with the use of various drugs. Beta antagonists is one of the commonly used drugs, amongst which the beta-1 adrenoceptor blocking agents (preferably Atenolol) are used for the treatment of hypertension. Like all other drugs, it exerts some side effects along with its beneficial pharmacological effects. It has shown some harmful effects on the male reproductive system and leads to change in the sperm morphology affecting male fertility. Our study was also conducted to observe the effects of atenolol and almond oil on the morphology of sperm.

Received on 24-12-2020
Accepted on 14-03-2021

MATERIAL AND METHODS

This laboratory-based randomized control trial was carried out between December, 2015 and July, 2016 by the Physiology Department of SCM, Islamabad. The laboratories who participated in this trial were CREAM Lab, which works under Army Medical College, Rawalpindi and laboratory of SIH, Islamabad. Ethical approval was taken from Shifa College of Medicine prior to the commencement of the study. One hundred and twenty male BALB-c mice were used in this clinical trial. The weight of these mice was between twenty-five to twenty-eight grams. A

controlled environment for these mice was maintained in National Institute of Health, Islamabad. By using non-probability convenience sampling, four random groups were obtained. National Institute of Health, Islamabad, formulated the diet given to the animals. Food and water were provided to the animals as per required. A temperature of 23±2 °C was maintained. They were equally exposed to light and dark for twelve hours. BALB-c adult male mice aging between 6-8 weeks and having weight between twenty-five to twenty-eight grams, having fully functional testes were part of this clinical trial. Mice having abnormal testes were excluded from the study. Animals in group A (n=30) served as Control and were given 1cc of water and 1cc of DMSO via oral route one time every day using a gavage needle for three months. In this clinical trial, the solvent used for atenolol was DMSO. Atenolol in the dose of 18 mg/Kg body weight/ml of DMSO via oral route one time given to mice in group B. It was administered every day for three months. Extract of almonds in the dose of 100 mg/Kg via oral route given to mice of group C. It was administered every day for three months. Atenolol in the dose of 18 mg/Kg body weight/ml of DMSO, and almond extract in a dose of 100 mg/Kg via oral route was given to mice of group D. It was administered every day for three months. Purchase of 5 milligram packing of Atenolol was made from Sigma, USA (Cat No. A-7655) and Prunus amygdalis (sweet almonds) from the local market. The extract was prepared by grinding almonds into a fine mixture and then dissolving it in water. After completion of 3 months, mice were anesthetized and dissected. Testes of mice were dissected out along with the vas deferens and epididymis. Fragments of tissue were removed when sperm solution was passed through a piece of clean muslin cloth along with addition of 0.5 ml of Eosin Y. Slides were prepared and examined for head and tail (short or absent tail) abnormalities of the sperms under the microscope under 40X. Focused slide under the microscope and then counted number of normal and abnormal sperms were counted in that particular focus, such three different views were focused under the microscope by moving the slide on left side only to avoid repetition of the same area and then dividing it by three, got the mean value and noted. Data analysed by SPSS-17. One-way ANOVA method and post-hoc test were applied to find out the statistical differences (p>0.05).

RESULTS

During the screening, the percentage of abnormal sperms (double-headed and short or absent tail) observed in this study was (1.580% ± 0.67%) in-group A (control) as presented in Figure 2. There was a significant increase in abnormal sperms found in-group B (Atenolol), in this group the abnormal sperms were (13.762% ± 5.31%). In-group C (Almond) abnormal sperms were found to be decreased (1.242%±0.793%) and in group D (Atenolol+Almond) abnormal sperms were increased (1.615 ± 0.96%) in comparison with mice in group A. The difference was merely significant. All these values are compared with that of control group in Table 1.

One-way ANOVA method and post-hoc test were applied to find out the statistical differences (p>0.05). ANOVA followed by post-hoc Tukey's test was also applied to compare the results of different groups with each other. This analysis is presented in Table 2for comparison of group B (Atenolol) with groups C (Almond) and D (Atenolol+Almond). This analysis showed that when group B (Atenolol) was compared with group C(Almond) and D(Atenolol+Almond), Percentage of double headed sperms and sperms with short or absent tail) also showed significant decrease in number of abnormal sperms in both groups C(Almond) and D (Atenolol+Almond). Comparison of group C (Almond) with group D (Atenolol+Almond) is presented in Table 3. This shows that Percentage of double headed sperms and sperms with short or absent tail was not significantly changed in two groups

Figure. 2: Comparison of sperm morphology (%age of double headed sperms and sperms with short or absent tail) among groups

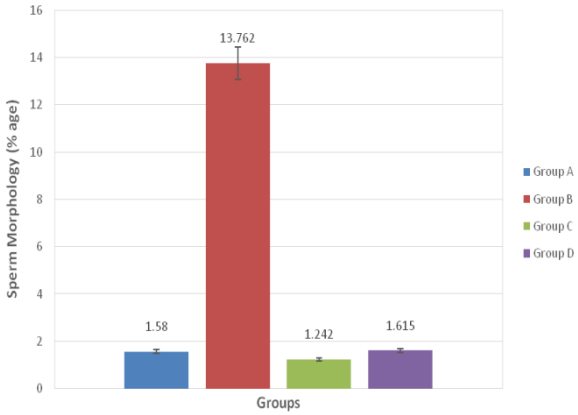

Table 1: Analysis of Variance (ANOVA) Pre (Post Hoc) test

Groups/ Variables	Group A (control) Mean Value± SD	Group B (Atenolol) Mean Value± SD	Group C (Almond) Mean Value± SD	Group D (Atenolol+ Almonds) Mean Value	P-Value
Percentage of double headed, short or absent tailed sperms)	1.580 ± 0.67	13.762 ± 5.31	1.242 ± 0.76	1.615 ± 0.96	0.000

All mean values are expressed as mean ± SD. * Significance (p < 0.05) at 95% Confidence Interval.

Table: 2: Comparison among variables of groups C and D with group B. Analysis of Variance (ANOVA) followed by post-hoc Tukey's-test

Groups/ Variables	Group B (Atenolol)	Group C (Almonds)		Group D (Atenolol + Almonds)	
	Mean value	Mean value	p- value	Mean value	p- value
%age of double headed sperms and sperms with short or absent tail)	13.762±5.31	1.242±0.76	0.000 *	1.615 ± 0.96	0.000*

All mean values are expressed as mean ± SD. *Significance (p < 0.05) at 95% Confidence Interval.

Table 3: Comparison of variables of group C with group D. Analysis of Variance (ANOVA) followed by post-hoc Tukey's-test

Groups/Variables	Group C (Almonds)	Group D (Almonds + Atenolol)	
	Mean value	Mean value	p- value
Percentage of double headed sperms and sperms with short or absent tail)	1.242 ± 0.76	1.615±0.96	0.953

All mean values are expressed as mean ± SD. *Significance ($p < 0.05$) at 95% Confidence Interval.

DISCUSSION

Our study was conducted to observe the effects of atenolol and almond oil on the morphology of sperm. According to our study, the morphology of sperm was altered by atenolol and the almond oil boosted those changes. The experimental groups were compared with the control group, as well as with each other.

Atenolol is a widely used beta-blocker in cardiovascular diseases but it has toxic effects on masculine fertility. El-Sayed et al conducted a study to see the effects on fertility of male rat by atenolol, metoprolol and propranolol. Rats were investigated following repeated oral administrations for 60 consecutive days. The increase in abnormalities of head and tail in sperms in this study are very similar to the findings of our study.

Martinez and Barthe conducted a study in which effects on different organs and endocrine glands were observed by injecting beta-blocker to adult male mice via intra-peritoneal injections. Necrosis and destruction of germinal cells of seminiferous tubules was an important change. This was an important beta-blocker toxic effect which is also shown by the results of our study because of the beta-blocking effect of atenolol.

Aruna and Krishnamurthy studied beta-blocking mutagenic effect in germ and somatic cells of Swiss albino rats. In erythrocytes, micronuclei were increased due to higher doses. No significant chromosomal damage was observed in germ cells. This shows that abnormalities in sperm morphology induced by administration of beta-blocker in our study are reversible, as beta-blockers do not cause mutations in a germ cell.

Qureshi et al conducted a study on brassica Rapa, Prunus amygdalus and zingiberOfficinale. These compounds aroused as aphrodisiacs in Arab Medicine. In their study, they observed acute toxicity test 24 hours where all these plants showed no toxicity. Chronic treatment was given to animals for three months. Different parameters regarding morphology and motility of sperm were observed. All these plants extracts significantly increased the sperm motility and sperm content without producing any spermatozoic effect. This effect produced by Prunus Amygdalus in this study conducted by Qureshi et al was similar to the effect produced in our present study. Qureshi et al observed the effects of Prunus Amygdalus 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.

According to the results of our study extract of Prunus Amygdalus was found beneficial for the reproductive system and it reversed all derangements caused by Atenolol, as the difference between values obtained from this and control were merely different. The mechanism of how atenolol causes these derangements is the decreased production of cAMP, which leads to a decrease in the production of testosterone. Testosterone is required for the normal functioning of testes (to produce sperms). When testosterone levels fall due to agents affecting testicular functioning, the abnormalities in sperms increase.

Almonds also contain Zn and arginine along with other amino acids. These ingredients may not actually antagonize the effects of atenolol, but via different mechanisms chemical constituents of Prunus Amygdalus improve the overall fertility parameters and through these changes, derangements produced by atenolol are reversed. Administration of antioxidants improves contralateral spermatozoid production and some other fertility parameters. Prepubertal animals can be maximally benefited by antioxidant treatment.

CONCLUSION

On basis of present study, it is obvious that use of Prunus Amygdalis has beneficial effects on sperms in mice. Moreover, it reverts atenolol-induced abnormalities in sperm morphology. So use of Prunus Amygdalis can be productive in treatment of male infertility.

REFERENCES

1. Bitok E, Sabate J. Nuts and Cardiovascular Disease. ProgCardiovasc 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 agerelated 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 (Agonoscenapistaciae) 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. ClinNutr 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. SocSci 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 Nutr2018;73:7–12.
9. Hajhashemi M, Rafieian M, RouhiBoroujeni HA, Miraj S, Memarian S, Keivani A, et al. The effect of Aloe vera gel and sweet almond oil on striaegravidarum 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 PediatrSurg2018;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 Nutr2018;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. ClinPerinatol 2019;46;173–85. Pak J Physiol 2019;15(4) http://www.pps.org.pk/PJP/15-4/Irum.pdf 23
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. 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. Martinez D, Barthe D. Histological study of the action of propranolol on the genital tract of the male rat. ActaAnat . 1981; 109: 346-54.
20. ArunaN, KrishnamurthyNBMutagenic evaluation of propranolol in somatic and germ cells of mice. Mutat Res. 1986; 173: 207-10.
21. 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.
22. Khan UA, Aslam M, Saeed SA . Effect of beta adrenergic antagonist on the production of testosterone by rat's Leydig cells. J Ayub Med CollAbbottabad 2004; 16: 26-8.
23. Macdonald AA, Stewart AW, Farquhar CM. Body mass index in relation to semen quality and reproductive hormones in New Zealand men: a cross-sectional study in fertility clinics. Hum Reprod. 2013; 28: 3178-87.
24. Moodley R, Kindness A, Jonnalagadda SB.Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia andwalnut) consumed in Southern Africa. J Environ Sci Health. 2007; 42: 585-91.
25. Ruggeri S, CappelloniM, Gambelli L, Nicoli S, Carnovale E. Chemical composition and nutritive value of nuts grown in Italy. Ital J Food Sci 1998; 10: 243-52.
26. Ribeiro CT, Milhomem R, De Souza DB, Costa WS, Sampaio FJ, Pereira-Sampaio MA. Effect of antioxidants on outcome of testicular torsion in rats of different ages. J Urol. 2014; 191: 1578-84.