ORIGINAL ARTICLE
EFFECT OF ALMONDS AND ATENOLOL ON SERUM LUTEINIZING HORMONE LEVEL IN MALE BALB-C MICE

Irum Rehman, Ifra Ashraf*, Shakir Khan**, Farheen Masood***, Aisha Naveed†
Department of Physiology, Margalla Institute of Health Sciences, Rawalpindi, *Shifa College of Medicine, Islamabad,
**Department of Biochemistry, ***Anatomy, †Medical Education, Margalla Institute of Health Sciences, Rawalpindi, Pakistan

Background: Along with other advantageous effects nuts are good for fertility and can improve derangements in different fertility parameters. With increasing incidence of hypertension leading to an increase in consumption of antihypertensives it is inevitable to depart their side effects. This study was designed to see the effect of administration of Prunus amygdalus, atenolol and combination of atenolol and Prunus amygdalus on level of serum luteinizing hormone (LH) in male Balb-C mice. Methods: It was an animal experimental study conducted in Physiology Department, Shifa College of Medicine, Islamabad, in collaboration with Shifa International Hospital, Islamabad, National Institute of Health, Islamabad and Centre for Research in Experimental and Applied Medicine (CREAM) Lab, Army Medical College, Rawalpindi from December 2013 to June 2014. After approval from Ethical Review Board, 120 mice which fulfilling inclusion criteria were divided into 4 groups of 30 mice each. Group A served as control group, group B mice were given atenolol, group C mice were given extract of almonds and group D mice were given atenolol and almonds. After the completion of 3 months, blood sampling was done and serum LH was measured. Data was analyzed on SPSS-17, Mean±SD was calculated for luteinizing hormone levels. One-way ANOVA was applied and p<0.05 was considered significant. Results: The mean value of serum LH in group B (Atenolol treated) was found to be significantly elevated. In group C (Almonds) serum LH was decreased but not significantly. Mean serum LH level in group D (Atenolol+Almond) was raised as compared to control group, but not significantly. Conclusion: Administration of Prunus amygdalis does not affect serum LH but atenolol causes a rise in serum LH.

Keywords: Prunus amygdalis, Atenolol, Luteinizing hormone

INTRODUCTION
Nuts are very important nutritious agents.1 During 37–past few decades a misconception developed about nuts and these were not considered as a component of healthy food.2 Extensive studies are being conducted to understand the beneficial and harmful effects of nuts in health. Prunus amygdalis commonly known as almond, is a focus of attention these days.3,4 The almonds have very unique composition comprising of monounsaturated fat, fibre, α-tocopherol, minerals such as magnesium and copper, and phytonutrients etc.5 Fat and fibre combination contributes to the cholesterol lowering effect of almond consumption, which causes a decrease in modifiable cardiovascular and diabetes risk factors such as body weight, glucose homeostasis, inflammation, and oxidative stress.6–8 Almond oil is studied for its beneficial effects on striae gravidarum and found helpful in this regard.9 It also has wonderful effects as sclerosing agent in treatment of rectal prolapse.10 Nuts and oil of Prunus amygdalis have important properties such as anti-stress, antioxidant, immunostimulant, laxative, fertility enhancer and other pharmacological actions.11–13

According to World Health Organization the incidence and complications of hypertension are increasing in developing countries.14 Treatment of hypertension is inevitable to avoid adverse effects of its complications on the quality of life. Besides lifestyle modifications various drugs are used for this purpose.15 Beta blockers are one of the effectively and frequently used drugs, amongst which the cardioselective agents like atenolol are preferred.16 Atenolol causes a rise in serum luteinizing hormone (LH).17 The present study was designed to observe the effects of Prunus amygdalis and atenolol on serum LH levels. Objective of the study was to compare the normal serum luteinizing hormone level in male BALB-c mice with level of LH after administration of Prunus amygdalus, atenolol, and combination of atenolol and Prunus amygdalus.

MATERIAL AND METHODS
It was randomized control trial on male BALB-c mice conducted from December 2013 to July 2014. After approval from the Ethical Committee the study was conducted 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 procured 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 groups (n=30) 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 were kept in 12 hour light/dark cycle.

The seeds of Prunus amygdalus (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\textsuperscript{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 a 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 was the control group. These mice were given 1 cc water and 1 cc DMSO orally once daily for 3 months with gavage needle. Group B mice were given atenolol in the dose of 18 mg/Kg body weight/ml of DMSO orally with gavage needle, once daily for 3 months\textsuperscript{18}. Group C mice were given extract of almonds in a dose of 100 mg/Kg orally with gavage needle, once daily for 3 months\textsuperscript{18}. Group D mice were 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\textsuperscript{18}. 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, intracardiac blood sampling of mice was done, and 1.5–2 ml blood was obtained from each mouse. Blood was transferred to the serum gel and clot activator tubes for collection of serum. These tubes were placed in a thermocool box containing ice packs till centrifugation. The blood was centrifuged for 10 minutes at 3,000 rpm.

After centrifugation, serum was pipetted out and stored at a temperature of -70 °C to -80 °C in CREAM Lab, Army Medical College, for estimation of serum luteinizing hormone level. Mouse luteinizing hormone ELISA Kit, Lot: C090207166, Cat No: CSB-E 12770m was used to measure the hormone.

Data were analyzed using SPSS-17. Mean±SD was calculated for luteinizing hormone levels. 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 serum LH level in all groups at the end of three months is presented in Table-1. The mean value of serum LH in group B was significantly elevated when compared with healthy controls of group A. Group C had decreased mean serum LH level compared to control group but the difference was not statistically significant. Mean serum LH level in group D was raised compared to control group but this was not significant.

The comparison of group B (Atenolol) with groups C (Almond) and D (Atenolol+Almond) is shown in Table-2. When group B was compared with group C and D, serum LH was found significantly decreased in group C though it was decreased in group D but difference was not statistically significant. When group C and group D were compared with each other, it was found that level of serum LH is increased after combined treatment of Almonds and Atenolol (D) as compared to Almond treatment only but this difference was not statistically significant.

Table-1: Serum LH level (IU/L) among groups (Mean±SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum LH (IU/L)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Control)</td>
<td>1.31±0.75</td>
<td>0.002*</td>
</tr>
<tr>
<td>Group B (Atenolol)</td>
<td>5.06±7.08</td>
<td>0.006*</td>
</tr>
<tr>
<td>Group C (Almonds)</td>
<td>1.10±2.39</td>
<td>0.809</td>
</tr>
<tr>
<td>Group D (Atenolol+Almonds)</td>
<td>2.30±3.97</td>
<td>0.704</td>
</tr>
</tbody>
</table>

\*Significant

Table-2: Serum LH level among groups (ANOVA followed by post-hoc Tukey's t-test)

<table>
<thead>
<tr>
<th>Group-wise comparison</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Control)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Group C (Almonds)</td>
<td>0.998</td>
</tr>
<tr>
<td>Group D (Atenolol+Almonds)</td>
<td>0.809</td>
</tr>
<tr>
<td>Group B (Atenolol)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Group C (Almonds)</td>
<td>0.069</td>
</tr>
<tr>
<td>Group D (Atenolol+Almonds)</td>
<td>0.704</td>
</tr>
</tbody>
</table>

\*Significant

DISCUSSION

We tried to highlight the effect of atenolol and almonds on serum LH independently and combined. This was done to see that whether intake of almonds has a positive effect on atenolol induced fertility derangements or not as was suggested that almonds are good aphrodisiac agent and improve different fertility parameters (hormonal and histologic), and Atenolol causes deterioration in different fertility parameters\textsuperscript{17,19}.

Weick\textsuperscript{19} conducted a study in ovariectomized adult female rats. He administered many drugs along with \( \beta \)-adrenergic receptor blockers. There was no influence on the pulsatile LH release. According to that study beta blockade has no effects on release of luteinizing hormone which is in contrast to the results of our study in which beta blockade increased release of luteinizing hormone, the difference might be due to the fact that Weick used a different beta blocker (propranolol), while we used atenolol a cardioselective beta blocker.

Al-Mehmood et al\textsuperscript{20}, demonstrated the effects of beta adrenergic system on release of LH. They concluded that the hypothalamic mediation of LH
release is through the activation of a beta 2-stimulatory component and the suppression of a beta 1-inhibitory component. The rise in LH under the effect of atenolol in that study was similar to our present study. However in that study rats were orchidectomized whereas in our study the activity of gonads for the release of testosterone was suppressed by atenolol.

Naveed et al determined the effects of a non-specific β-adrenergic receptor blocker on luteinizing hormone in male rats. Serum luteinizing hormone assay showed significantly lower serum luteinizing hormone level in 4 mg treated rats when compared with control and 1 mg treated rats. Serum level of both hormones was reversible after a recovery period of sixty days. These results were in contrast with our present study. The reason for this contrast may be because they used propranolol which is a non-selective beta blocker and we used Atenolol which is a specific beta-1 blocker. However, our results are consistent to study conducted by Al Mehmood et al who used selective beta-1 blocker. If beta receptors are blocked non-specifically then these decreased LH levels and if selective beta-1 blocker is used then it increases the levels of LH.

In the study conducted by Nusier et al on beta blocker and its effects on reproductive function in adult male mice, serum LH was decreased in male mice in response to beta blocker, this was in contrast to the results of our study in which serum LH level is increased after administration of beta blocker, this difference may be because of selectivity of the beta blocker used or because of difference in dosage and breed of mice.

Mukhtari et al observed the effects of alcoholic extract of nuts (walnut) on FSH, LH and testosterone concentration in adult male rats. According to results of that study, alcoholic extract of Walnut affected pituitary testis axis and increase GnRH and LH secretion rate, therefore increased reproductive activity in male rats. In our study extract of nut (Prunus amygdalis) did not significantly alter the levels of LH, the cause of this difference was probably the difference in the nut used and the animal species.

El-Khamisy et al conducted a study on nuts (hazelnut). Their study was aimed to explore the hypoglycemic effect of hazelnut and its effect on some sex hormones in diabetic female rats. Feeding supplemented diet with hazelnut at the three different levels caused significant decrease in concentrations of insulin, thyroid stimulating, follicle-stimulating and luteinizing hormones as compared to that of untreated diabetic rats. That study concluded that nut intake reduces the level of LH. Our findings were different from their study; there was no significant effect on serum LH levels. The probable cause of this difference is the difference in the nut used.

CONCLUSION
On basis of the results obtained in this study, it is concluded that administration of Almonds does not significantly affects serum LH but atenolol causes a significant rise in serum LH.

ACKNOWLEDGMENT
The authors gratefully acknowledge the support of Shifa College of Medicine, Islamabad, National Institute of Health, Islamabad and Centre for Research in Experimental and Applied Medicine (CREAM) lab, Army Medical College, Rawalpindi.

REFERENCES


Address for Correspondence:
Dr. Irum Rehman, Assistant Professor, Physiology Department, Margalla Institute of Health Sciences, Rawalpindi, Pakistan. Cell: +92-321-7826878
Email: junaidirum@gmail.com

Received: 3 May 2018  Reviewed: 12 June 2018  Accepted: 12 June 2018