ORIGINAL ARTICLE

Evaluate the Effect of Amlodipine Besylate on Testosterone and Fertility of Male Albino Rats

Rahimullah Rahi[1], Shahid Ali[1], Abdullah Jan[2], Syed Azhar Hussain Zaidi[1], Zubaida Anwar[1], Sumaira Deen Muhammad[1]
[1]Bolan University of Medical and Health Sciences, Quetta, [2]Loralai Medical College, Quetta, Pakistan.

ABSTRACT

Background: Amlodipine is a Ca^{++} channel blocker, routinely used for treatment of hypertension. The mechanism underlying its anti-hypertensive effect is dilatation of blood vessels. Testosterone, the hormone produced by testis, is responsible for development of male sexual secondary characteristics. The aim of study was to find out the effect of Ca^{++} channel blocker, Amlodipine besylate on serum testosterone level in male albino rats.

Methods: Duration of study was 30 days and conducted in department of pharmacology, 24 male Albino rats were grouped into 4 groups (each having 6). Group A was control group, Group B, C and D were treated with Amlodipine Besylate drug at doses 0.07, 0.14 and 0.28mg/kg body weight respectively (Suspension of 5 mg tablet of Amlodipine dissolved in 10 ml distilled water prepared and further diluted to 1:10ml with distilled water). Independent t test and ANOVA with Tukey's test were used for comparison of quantitative variables with in groups.

Results: Serum testosterone level reduced in groups treated with Amlodipine. Serum testosterone level of control group is 11.22 while group B, C and D were 4.7, 2.6 and 0.7 respectively. Serum FSH level of control group is 0.10±0.01 while group B, C and D were 0.24±0.35, 0.34±0.40 and 0.08±0.03 respectively. It showed that increasing the dose of Amlodipine markedly reduce testosterone level and increases FSH level in male albino rats.

Conclusion: Amlodipine severely reduce level of serum testosterone (p=0.001) and increases FSH level (p=0.305) which can lead to infertility in male albino Rats.

Keywords: Amlodipine; Testosterone; Infertility.

Corresponding Author:
Dr. Shahid Ali
Bolan University of Medical and Health Sciences,
Quetta, Pakistan.
Email: drshahidali2009@gmail.com
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INTRODUCTION

The albino rat used in laboratory, Rattus norvegicus albinus, belongs to family Muridae and order Rodentia. These stocks were reproduced at the Wistar Institute in 1906 for study of neuro-behavioral sciences, neuro-anatomy, nutrition, cancer, toxicology, genetics, endocrinology and other scientific research[1]. At about 2 months of age, male and female rats become sexually mature. Breeding begins at about three months of age. Fertility is at maximum 100-300 days of age[2]. The testis is the Latin word derived from testicles which means "witness" of virility (manhood), it is the male gland which is helpful for both reproductive (exocrine gland that generates male germ cells (spermatozoa) and have endocrine functions that releases an important hormone testosterone[3]. In 1850 German histologist, Franz Leydig first time described Leydigcells[4]. These cells secrete testosterone[5]. Seminiferous tubules produce about 20,000,000 male reproductive cells a daily called as spermatozoa. Each testis contains about 250-1000 seminiferous tubules that measure about 30-70cm in length and 150-250µm in diameter[6]. The testis of males produces greater than 95% of testosterone in human being. Testosterone is secreted by Leydig (interstitial) cells[7]. In the tissues, testosterone converts into its active metabo-

lite dihydrotesto- sterone[8].

Theca cells of the ovaries also synthesize small amount of testosterone in women, cortex of adrenal gland (zona reticularis), placenta and skin in both sexes synthesize testosterone[9]. At puberty it promotes secondary sexual characteristics such as bone mass, increased muscle mass, deepening of voice, body hair growth, coagulation, hematopoiesis, psychosexual, cognitive behavior, lipid, protein and carbohydrate metabolism[10]. Testosterone is beneficial for health and maintains well-being[11]. After secretion by the anterior pituitary gland the FSH stimulates the sertoli cells, without this the conversion of spermatids to spermatozoa wills not occur[12]. Elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are seen in diagnosed cases of azoospermia [13].

It is estimated that approximately 1 billion people worldwide have hypertension, which contributes to 92 million lifelong disabilities and more than 7.6 million deaths per year. It is also evaluated that 20% of adults worldwide have hypertension at some stage of life. About 65.4% of individuals have hypertension in this age group[14]. Prevalence of hypertension in Pakistan is about 50 percent in population. Above 50 years of age, it is around 30 percent in people at 30 years age and 5 to 7 percent among the children[15]. One of the common causes of adult male infertility is impaired or blocked calcium signaling[16]. Therefore, it is logical to say that if calcium ions entry inside the cell blocked would affect libido, hormonal secretion and fertility[17]. Dr. Albrecht Fleckenstein introduced calcium channel blockers also called antagonists in 1964 at Freiburg University of Germany[18]. They are routinely prescribed drugs for different cardiovascular disorders[14,19] and cause vasodilation, by these mechanisms it decreases blood pressure[20]. Dihydropyridines have strong vasodilating and good cardiac effects they are most vasoselective class of Calcium channel blockers[21] and greater sensitivity to dihydropyridines[22]. Amlodipine used orally in adult male rats for 1 month, markedly reduce serum testosterone levels and spermatogenesis[23,24]. Amlodipine if used for prolong period of time cause few non-mobile sperms in semen analysis of men, it may also cause azoospermia[25].

To determine the infertility effects of 0.07, 0.14 and 0.28 mg/kg body weight of Amlodipine and compare the infertility effects of Amlodipine drug in three different dose groups in adult male albino rats. The dose calculated from the study by Shannon Reagon-Shaw et al[26]. The study aimed to investigate the effect of Amlodipine on serum testosterone. As study was already conducted by using different methods and doses but based on these doses no study was conducted in the past.

METHODS

The study was conducted in Basic Medical Sciences Institutes, pharmacology Department, Jinnah Postgraduate Medical Centre (JPMC)-Karachi. From multinational pharmaceutical company Amlodipine besylate tablet of 5 mg batch number 1505003 was purchased. Amlodipine tablet of 5 mg grinded and mixed in 10 ml distilled water to make suspension. This suspension of 1ml mixed in 10 ml distilled water. Therefore, 1 ml of this suspension contains 0.05 mg of Amlodipine and used orally once a day as 0.07, 0.14 and 0.28 mg/kg body weight for thirty days. Dose can be calculated as 5mg/10ml=0.5mg/ml, 0.5mg/10ml=0.05 mg/ml. Twenty-four male albino rats of adult age (3-4 months) were taken for this study. Weights of animals lie between 200 to 320 grams. Twenty four animals were divided into 4 equal groups i.e. 6 animals in each group.

Group A (Control) animals were kept on normal diet only[27]. Before starting the research, male albino rats were kept for one week at room temperature for acclimatization. Animals were weighed at day 0 for drug dose purpose and different markers marked each group. According to their groups, they were kept in four cages and marked as A, B, C and D groups for identification purpose. Animals were observed weekly for their diet and physical activity. After one week each group of rats were treated with their respective dose of Amlodipine, but group A (control) was kept only on normal diet. After the completion of study period i.e. 30 days, all the animals were dissected after giving ether anesthesia. Fingers, which were beating, palpated the heart of the rat and five ml of blood was drawn from all rats by puncturing heart with the help of 5 ml disposable syringe for estimation of testosterone concentration in serum.

The serum testosterone level can be calculated by electrochemiluminescence (ECL) immunoassay method Catalogue Number: 05200067190 Cobas e 411 (rat/mouse ECLIA kit (ROCHE, SWITZERLAND, FDA approved, C/E marked)[28]. The blood samples were centrifuge to get serum, which were collected after animals sacrificed. Blood samples were stored at -80°C. Before use all, the samples were brought to room temperature. Following Reagents - Working Solutions used are M-Streptavidin-coated micro-particles (transparent cap), R1Anti- testosterone-Ab~biotin (gray cap) and R2Testosterone-peptide~Ru(bpy) (black cap). Data was entered and subjected to descriptive analysis using SPSS. Further ANOVA with Tukey/independent t-test were used for comparison of quantitative variables with in groups. A p-value of <0.05 was considered as statistically significant.

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RESULTS

The albino rats of group B, C and D were administered Amlodipine besylate daily at doses 0.07, 0.14 and 0.28 mg/kg body weight orally for 30 days respectively. This showed decrease in mean serum testosterone level i.e. 4.79± 0.57, 2.61±0.71 ng/ml and 0.74± 0.51 ng/ml respectively as compared to control (Group A) i.e. 11.22± 1.91ng/ml. Highly significant p-value, 0.001 is shown in Figure 1. A p-value of <0.05 was considered as statistically significant.

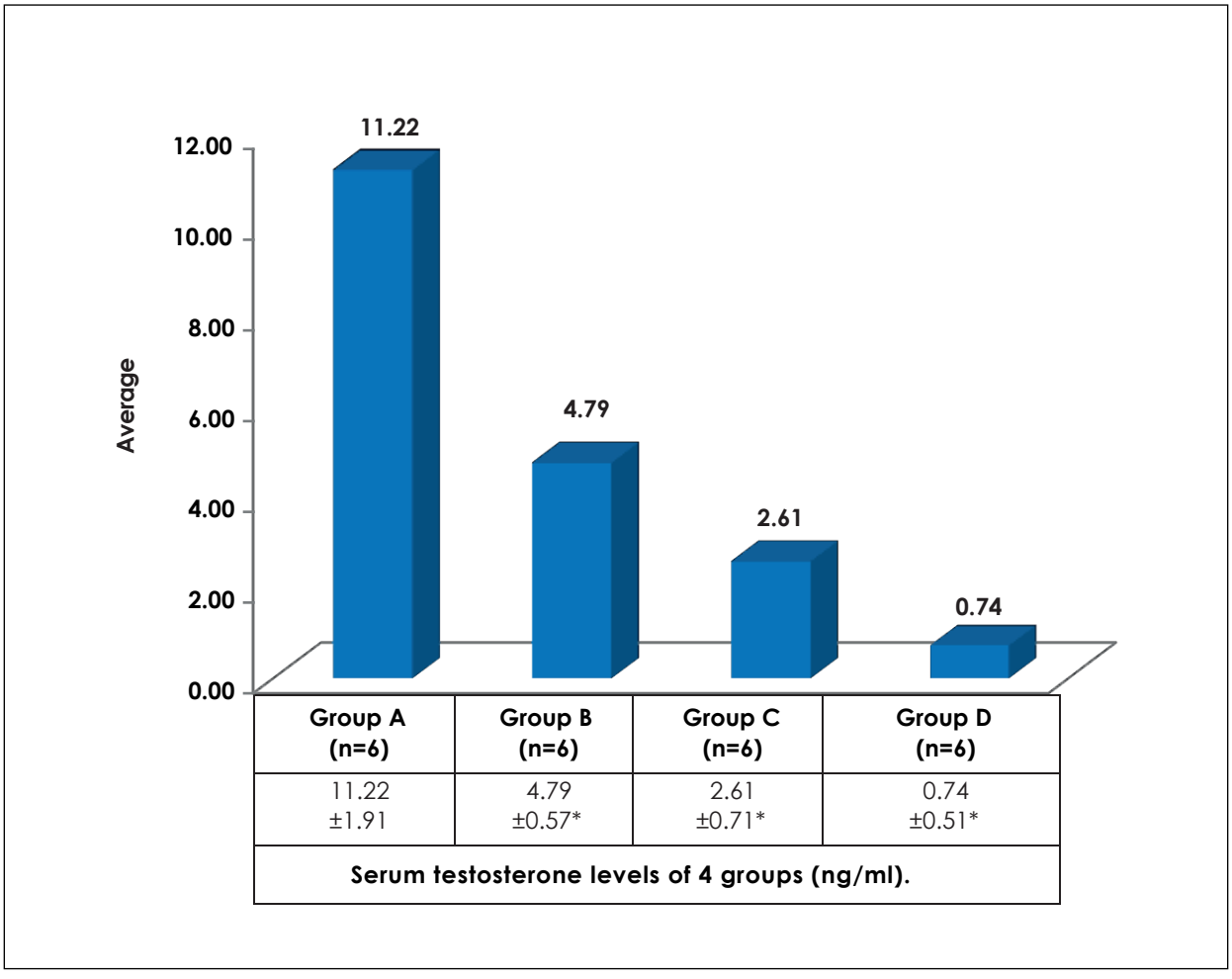

Figure 1: Serum testosterone levels of 4 groups (ng/ml).

When serum testosterone level of Group A (11.22±1. ng/ml) was compared with Group B (4.79±0.57ng/ml) p - value, 0.001 was highly significant. A highly significant p - value of less than 0.001 was found when serum testosterone level of Group A (11.22±1.91ng/ml) was compared with Group C (2.61 ± 0.71ng/ml).

On comparison of serum testosterone level of Group A (11.22±1.91ng/ml) with Group D (0.74 ± 0.51 ng/ml) p - value, 0.001 was highly significant. p - value, 0.012 was significant when serum testosterone level of Group B (4.79± 0.57ng/ml) was compared with Group C (2.61±0.71ng/ml). When serum testosterone level of Group B (4.79± 0.57ng/ml) compared with Group D (0.74±0.51ng/ml), it shows highly significant p-value (0.001). Significant p-value of 0.034 obtained by comparing serum testosterone level of Group C (2.61± 0.71ng/ml) with Group D (0.74±0.51ng/ml).

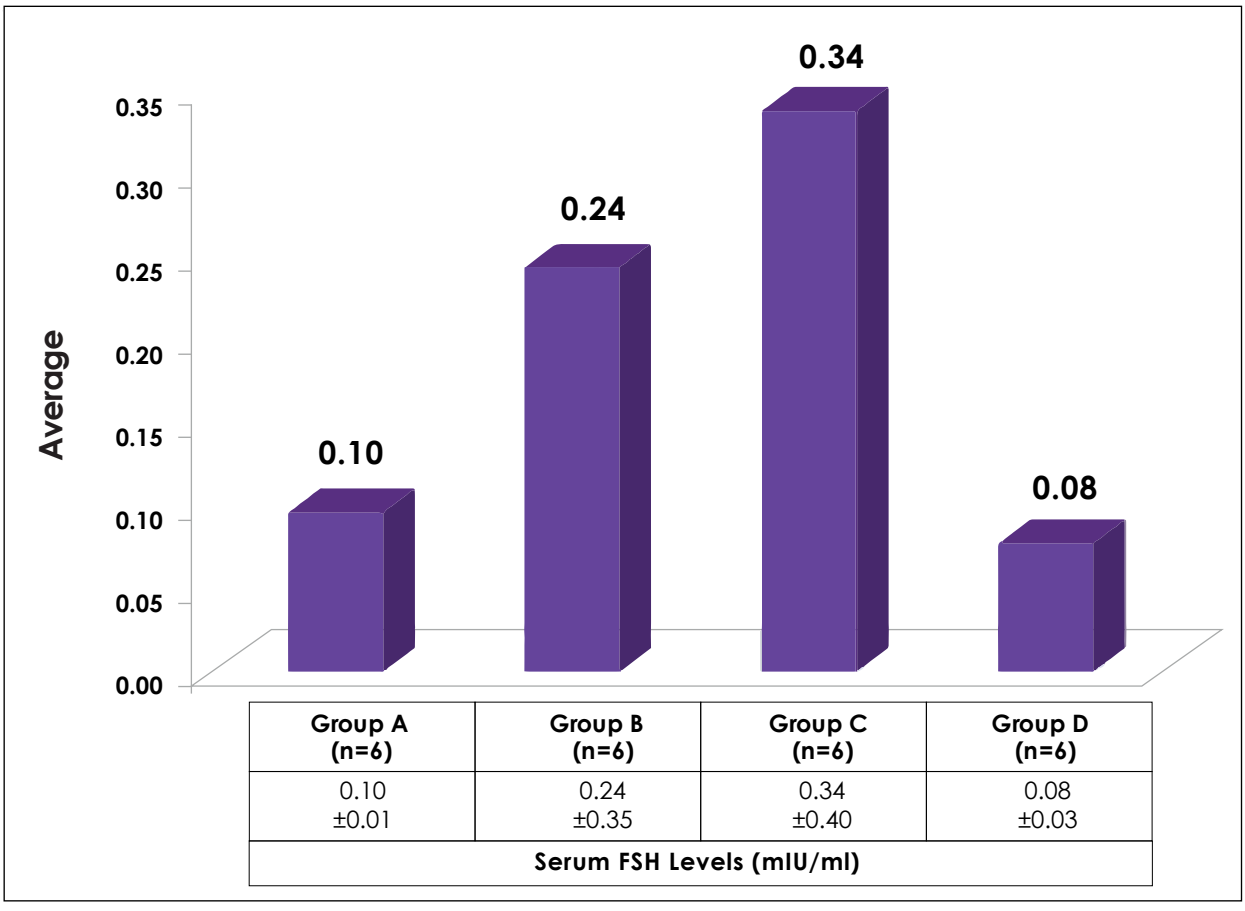

Figure 2: Comparison of serum FSH levels (mIU/ml).

No significant difference was observed (p>0.05). The comparison of serum FSH levels of Group A (0.10 ± 0.01) with Group B (0.24±0.35) was done. On statistical analysis p-.value of 0.775, was non-significant, as shown in table 13 and Figure 2.

DISCUSSION

Hypertension, also called as raised blood pressure. It is a condition of persistently raised pressure in the blood vessels actually it is the pressure of circulating blood on the walls of blood vessels. Most of this pressure occurs due to work done by the heart by pumping blood through the circulatory system. It is also said that "blood pressure" refers to the pressure in large arteries of the systemic circulation. Blood pressure is usually measured in terms of the systolic pressure (it is the maximum pressure during one heartbeat) and diastolic pressure (it is the minimum pressure in between two heartbeats) and it can be calculated in millimeters of mercury (mmHg), above the surrounding atmospheric pressure. Blood distributes from the heart to different parts of the body through vessels. The force of blood pushing against the walls of blood vessels (arteries) as the heart pumps it generates blood pressure. Harder the heart pumps the higher will be the pressure[29].

Increased blood pressure variability is linked with development and severity of cardiovascular and kidney damage and with an increased risk of cardiovascular events (myocardial infarction, myocardial ischemia and stroke) and these all contribute to elevated mean blood pressure above the normal levels[30].

Amlodipine besylate, a calcium channel blocker drug and sold as brand name Norvasc. It is a drug used for the treatment of high blood pressure and coronary artery disease, used since decades for hypertension; it causes vasodilatation by relaxing peripheral vessels and reduces peripheral resistance, which ultimately decreases blood pressure. Lowering of blood pressure reduces the risk of fatal and nonfatal cardiovascular events, myocardial infractions and strokes being a Ca^{++} channel blocker it is not typically recommended for heart failure, if other medications fail to treat high blood pressure or heart-related chest pain then Amlodipine may be used. It is usually taken orally and has half-life of about 24 hours. Following are Common adverse effects of Amlodipine feeling tired, swelling, nausea and abdominal pain. Serious side effects include reduced blood pressure and heart attack. It is unclear that whether use in pregnancy or during breast-feeding is safe.

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The present study was designed to evaluate the anti-reproductive and infertility effects of the drug Amlodipine besylate in rats. Amlodipine besylate was given orally and daily for thirty days to the adult male albino rats (n=24) divided into four groups. Current study corresponds to study conducted by Onwuka, in which Amlodipine was administered to the same groups (B, C & D) by the dose of 0.01, 0.02 and 0.03 mg/kg body weight respectively for 6 weeks, also showed decrease in serum testosterone level.

Results of current study i.e. decrease in serum testosterone levels also supports the study conducted by Rabia et al., in which same drug was administered to adult male rats (Sprague Daley) at the dose of 0.14 mg /kg body weight orally and daily for 50 days also showed decreased in serum testosterone levels and testicular weight. The results of hormonal profile of our study showed that Amlodipine caused decrease in serum testosterone, increase in serum FSH level in adult male rats are in accordance with the study conducted by Webb and colleagues. They narrated that low level of testosterone and high levels of FSH and LH are seen in hormonal profile of human males diagnosed as Azoospermia (sperm count zero). While low level of serum testosterone and high level of FSH are seen in hormonal profile of diagnosed cases of Oligozoospermia (sperm count less than 20 million/ml).

CONCLUSION

Amlodipine, a Ca^{++} channel blocker, reduces fertility in male Albino Rats by reducing serum testosterone level and increasing FSH level at three different doses. Thus, long-term use may lead to impotency and infertility in adult males.

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CONFLICT OF INTEREST

No conflict of interest found among the authors.

ETHICS APPROVAL

The ethics committee of the Bolan University of Medical and Health Sciences has approved the study.

AUTHORS' CONTRIBUTION

AJ and SAHZ helped in writing of article, ZA did important task of initiation of relevant literature. SA and SD performed task of writing the discussion of the article. RR did his contribution in analysis of the article.

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