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RESEARCH ARTICLE
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THE EFFECTS OF AMLODIPINE ON SERUM TESTOSTERONE - A MULTICENTER STUDY IN PAKISTAN

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ABSTRACT

Background: Amlodipine is one of the several calcium channel blockers (CCBs) used to treat cardiovascular diseases. Some research indicates that CCBs may affect male reproductive health by changing testosterone levels, even though their effects are mostly cardiovascular. In Karachi and Quetta, Pakistan, this study uses an animal model to examine how amlodipine affects serum testosterone levels.

Methods: A total of 50 male animals (rats and rabbits) were randomly divided into four groups: a control group (no amlodipine) and three experimental groups receiving low, medium, and high doses of amlodipine. The drug was administered orally over 4–6 weeks. Serum testosterone levels were measured before and after treatment using the enzyme-linked immunosorbent assay (ELISA) method. Data were analyzed using paired t-tests and ANOVA and Correlation to assess statistical significance.

Results: There was no statistically significant difference in testosterone levels before and after amlodipine treatment ($p = 0.068$). Additionally, testosterone levels did not significantly vary among different dosage groups ($p = 0.732$), and the correlation between amlodipine dosage and testosterone levels was weak and statistically insignificant ($r = -0.082$, $p = 0.570$). Although 50% of the animals exhibited changes in reproductive behavior, no corresponding hormonal fluctuations were detected.

Conclusion: Amlodipine had no discernible impact on serum testosterone levels in the animal models used in the investigation. These results are in contrast to some earlier research that found that

administering CCB decreased testosterone levels. Differences in experimental settings, drug delivery length, or species variability could be the cause of the disparity. To be certain whether amlodipine affects male reproductive health, more research is required, especially clinical investigations involving human patients.

Keywords: Amlodipine, Calcium Channel Blockers, Serum Testosterone, Male Reproductive Health, Hormonal Effects,

INTRODUCTION

Calcium channel blockers (CCBs), which block calcium channel transmembrane influx, are the most recommended medications to the treatment of cardiovascular disorders. Calcium ions are used in many biological processes, including excitation contract coupling, excitation secretion coupling, mitosis, fertilization, and control of gene expression. The effects of CCBs though are mainly confined to heart and vascular smooth muscles. Heterogeneity of calcium channels or a 'use dependence' feature of CCBs (Triggle DJ, 2007) causes this tissue selectivity from the level of heterogeneity of calcium channels which blocks the most active calcium channels more effectively. However, according to reports, there exist these cases of infertility in male CCB consumers, despite gigantic cardiovascular selectivity (Benoff S, 1998; Nowak, R, 1994). So further research is needed to find out how the precise role that CCBs play in infertility.

Calcium ions significantly influence the manufacture, release, secretion as well as the action of hormones by regulating their manufacture, release, as well as its action on target tissues. Since calcium antagonists should prevent calcium from entering the cell, this activity should also be influenced. Nevertheless, calcium channel blockers have served well as agents to treat angina and hypertension, being effective because they lower peripheral vascular resistance and decrease blood pressure by blocking calcium transmembrane influx in vascular smooth muscle cells (CLEMENT DL et al., 1994). In spite of this strong need of vascular specificity, many calcium channel blockers like verapamil, diltiazem, nifedipine, and lecideine were observed to be able to interfere in hormonal output (mainly pituitary) (26–29). It has also been shown that rats (22) as well as humans (27) treated with verapamil and diltiazem have lower testosterone levels.

This amlodipine known also as besylate, mesylate or maleate is a long-acting calcium channel blocker (dihydropyridine) used to treat angina and to lower blood pressure. Amlodipine works like other calcium channel blockers by not allowing calcium to pass through the calcium channel into the transmembrane space and thereby preventing Ca mediated smooth muscle contraction of the wall of the artery that results in decreased peripheral resistance and consequently lowered blood pressure (Frick MH et al., 1988). In angina it helps to increase blood flow to the heart muscle (Choi SM et al., 2009). The typical functions of most biological processes require calcium (Glasser SP et al., 1988). Most of the effects of calcium channel blockers are to the heart and vascular smooth muscles. Reports suggest that often calcium-channel blockers may cause anti reproductive effects in males with long term use even though they are very selective in their cardiovascular effects. The issues of sexual function have been known to have an impact on management of hypertension and possibly on discontinuation of medication and final treatment plans. The medication of Mild Hypertension Study (Garko B et al., 2005) (TOMHS) allows for sexual function examination and looks at impact of medication on sexual function in men and women attending stage I diastolic hypertension. The double blind, randomized controlled experiment called TOMHS gave acebutolol, amlodipine maleate, chlorthalidone, doxazosin maleate, or enalapril maleate to 902 patients with hypertension. Although the values of long-term incidence of erection issues in hypertensive males treated by chlorthalidone were low, data in this study showed higher incidence on long term. There are also low rates of sexual issues in the Women with hypertension report, irrespective of type of medication. Patients that experience decreased sexual function from the use of antihypertensive agents are more likely to discontinue treatment and to have a reduced quality of life (Ferrario CM et al., 2008; Grimm RH Jr et al., 1997).

Testosterone is one of the steroid hormones of androgen group. While the adrenal glands secrete small amounts of testosterone, the primary sites in mammals are the testes of males of which the ovaries of female mammals. It is an anabolic steroid but the main male sex hormone.

Testosterone is the primary hormone for male reproduction, and hence a review or literature indicates both conflicting treatment on the serum testosterone levels in CCBs (Almaida SA et al., 2000 and Albers MM et al., 1991). Additionally, since these CCBs have also been shown to have an antiproliferative quality (Stepien O et al., 1997; Tang J et al., 1997), it would seem feasible to envision that CBs do prevent continuous proliferation of various testicular cells as well as compromise reproduction.

Maintaining good health and avoiding osteoporosis is entirely dependent on the amount of testosterone present in one's body. Even with the fact that the body of an adult male makes forty to sixty times as much testosterone as an adult female, females are more responsive hormonally than anatomically or biologically (Dabbs, M. et al., 2000). Andropause, a disease caused by a slow decline in testosterone levels in a man during middle age that reduces the capacity to enjoy sex and have high quality erections (Heaton JP, 2003; McCulloch A, 2003), also affects his ability. There are also mood and emotional changes, especially in men who have decreased testosterone, in addition to erectile dysfunction with loss of sexual desire. However, the ranges of the males and females overlap at the low and high ends, respectively, without overlap.

RESEARCH OBJECTIVE

The aim of this study was to determine the effect of amlodipine on blood testosterone levels in animals' models in a Karachi and Quetta, Pakistan tertiary care centers. The purpose of the experiment is to examine the potential changes in testosterone levels after amlodipine treatment with a controlled experimental methodology. The findings will help researchers learn more about how the medication affects endocrine activity and provide such information that could be useful for future clinical trials and human studies.

RESEARCH QUESTIONS

1. Does amlodipine affect serum testosterone levels in animal models?
2. Is there a significant difference in testosterone levels before and after amlodipine administration?
3. Does the dosage of amlodipine influence changes in serum testosterone levels?

LITERATURE REVIEW

There are many studies that looked at the effect of calcium channel blockers on the reproductive hormones. Nassar et al (2015) have reported in their study that long term calcium channel blockers usage will lead to decrease of testosterone levels in males. The hypothesis is that the underlying mechanism is the reduction of the calcium dependent enzymatic activity in the Leydig cells [that control testosterone synthesis] (Smith et al., 2017). That disturbance may produce a diminished fertility, and a changed spermatogenesis.

Amlodipine is a frequently prescribed dihydropyridine calcium channel blocker and also used for treatment of angina and hypertension due to its effectiveness in preventing transmembrane calcium influx in coronary and vasculature smooth muscle tissue. Decades have known about its cardiovascular benefits, but new research reveals that it can have a hurtful effect on male reproductive health, mostly on testicular function and serum testosterone levels.

A few animal experiments have examined the treatment of male reproductive parameters by amlodipine. Latif et al. (2008) investigated the effects of amlodipine at a level of 0.14 mg/kg per day in 50 days in adult male Sprague-Dawley rats. It was found that blood testosterone level, absolute testicular weight and gonado somatic index of the treatment group was significantly smaller than that of the control group. This indicates that exposure to amlodipine for the extended period causes testicular regression and decrease in testosterone production.

Onwuka et al. (2016) also studied the effect of different amlodipine dosage (0.01, 0.02 and 0.03 mg/kg body weight) on male albino rats for six week. On the dose dependent manner, the data showed that amlodipine increases the suppression of testosterone synthesis.
Jamil et al. (2020) also supplied further credence to these findings, as they examined the reproductive effects of amlodipine besylate and the male albino rats. Their study showed that over 30 days, when they gave amlodipine orally, serum testosterone was reduced and serum FSH was increased. Changes in these hormones are a symptom of possible problems with fertility and also reduced testicular function. Clinical findings are, in fact, added to these animal research. In male hypertension patients treated with the amlodipine medication, serum testosterone levels were depressed as reported by Al-Shebli (2017). Study showed that amlodipine medication could decrease sex hormone levels in men and that this effect is dose dependent and especially with aging.

HYPOTHESES

❖ H1: There is a significant difference in testosterone levels before and after amlodipine administration.
❖ H2: The dosage of amlodipine does not significantly impact changes in serum testosterone levels.

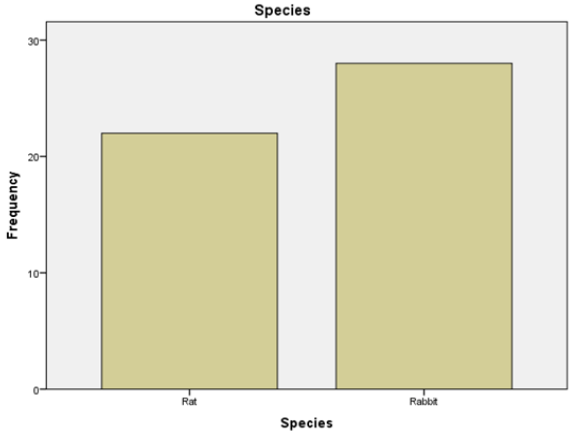
METHODOLOGY

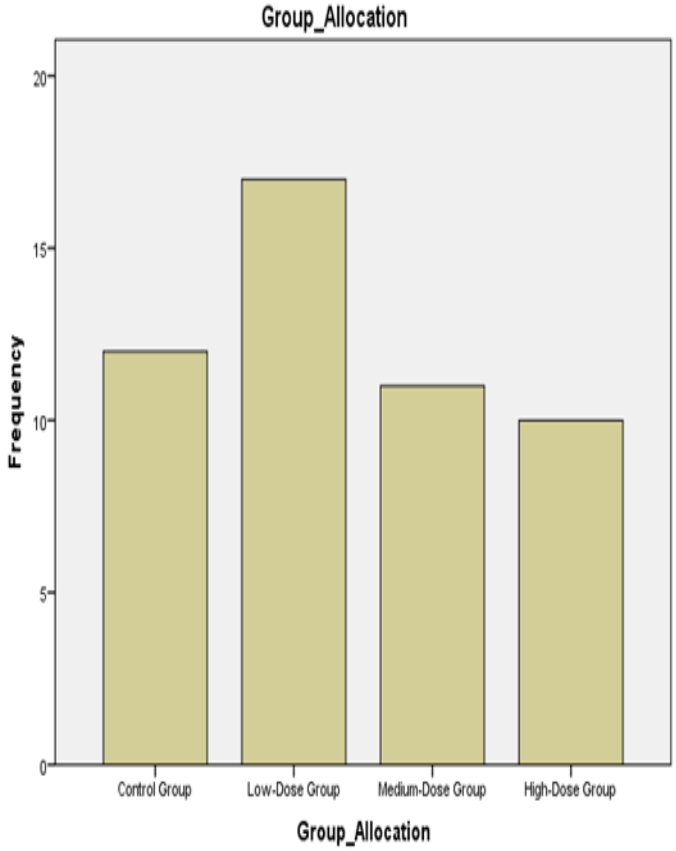
The aim of this experimental investigation is to find out the effect of amlodipine on the blood testosterone levels in animal model at a tertiary care hospital of Karachi and Quetta, Pakistan. To prevent only healthy subjects without underlying hormonal disorders to participate, I will select 100 male animals with strict inclusion and exclusion criteria. Third, the animals will be randomly assigned to four groups: three experimental groups will receive low, medium and high dosages of amlodipine, and a control group will not be given amlodipine. Carefully under monitored circumstances, the medication will be given either orally for four to six (4-6) weeks.
In the case of amlodipine, baseline serum testosterone levels will be measured before treatment and at the end of the trial period to determine the impact of treatment. Venipuncture will be used to obtain blood samples and the testosterone levels will be assayed using the enzyme linked immunosorbent assay (ELISA). Physiological changes will be documented by hormones, body weight, reproductive behavior and general health. SPSS software will be used to make data analysis. Descriptive statistics will be used to summarize testosterone levels and differences between groups will be identified using paired t tests or ANOVA. Statistical significance is said to be when a p value is less than 0.05.

RESULTS

Table 1: Categories of Species

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Rat	22	44.0	44.0	44.0
	Rabbit	28	56.0	56.0	100.0
	Total	50	100.0	100.0	





The animal species included in the study are categorized in this table. 28 (56%) were rats and 22 (44%) of the 50 subjects were rabbits. By doing this, the study's species distribution for comparison analysis is guaranteed to be balanced.

Table 2: Group Allocation

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Control Group	12	24.0	24.0	24.0
	Low-Dose Group	17	34.0	34.0	58.0
	Medium-Dose Group	11	22.0	22.0	80.0
	High-Dose Group	10	20.0	20.0	100.0
	Total	50	100.0	100.0	

The distribution of animals among the various study groups is displayed in this table: Twelve animals (24%) in the control group were not given amlodipine. Low-Dose Group: A tiny dosage was given to 17 animals (34%). 11 animals (22%) in the medium-dose group were given a moderate dosage. Ten animals (20%) in the high-dose group were given a high dosage.
This distribution guarantees a comparative evaluation of the effects of different amlodipine dosages on blood testosterone levels.

Table 3: Weight Change During the Study

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	increase	19	38.0	38.0	38.0
	decrease	22	44.0	44.0	82.0
	No change	9	18.0	18.0	100.0
	Total	50	100.0	100.0	

The effect of amlodipine on body weight is shown in this table: The weight of 19 animals (38%) increased. Twenty-two animals (44%) lost weight. There was no discernible change in weight in 9 animals (18%). The findings suggest that amlodipine might affect metabolism or general health, which calls for more research on its physiological impacts.

Table 4: Change in Reproductive Behavior

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	25	50.0	50.0	50.0
	No	25	50.0	50.0	100.0
	Total	50	100.0	100.0	

Changes in reproductive behavior are shown in this table: Twenty-five animals, or 50%, showed alterations. Twenty-five animals, or 50%, showed no behavioral changes.
The necessity to investigate differences in testosterone levels is further supported by the possibility that behavioral changes could be a sign of hormonal disturbances.

Table 5: Paired T-Test (Pre- and Post-Treatment Testosterone Levels)

	Mean	St. Dev	95% Confidence interval Lower	Upper	t	df	Sig(2-tailed)
Baseline Testosterone- Post Treatment Testosterone	0.66920	2.53764	-0.5199	1.39039	1.865	49	.068

This test evaluates whether there is a significant difference in serum testosterone levels before and after amlodipine administration.
If P-value or Significance value is less than 0.05 then reject the hypothesis, Since the p-value (0.068) is greater than 0.05, we fail to reject the null hypothesis. This means that there is no statistically significant difference in serum testosterone levels before and after amlodipine administration at a 5% confidence level. Thus, Hypothesis H1 ("There is a significant difference in testosterone levels before and after amlodipine administration") is not supported.

Table 6: One-Way ANOVA (Analysis of Variance)

ANOVA

Post_Treatment_Testosterone_ng_dL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.375	3	1.792	.431	.732
Within Groups	191.054	46	4.153		
Total	196.429	49			

*We compare **testosterone levels** across multiple **dose groups**. *
This test assesses whether different doses of amlodipine lead to significant variations in testosterone levels.
If the ANOVA test results show a p-value less than 0.05, it would indicate significant differences in testosterone levels among dose groups. If $p > 0.05$, it means that dosage variations do not significantly impact testosterone levels. Since P-value is 0.732 and its greater than 0.05 then Hypothesis H2 ("The dosage of amlodipine significantly impacts serum testosterone levels") will also not be supported.

Table 7: Correlation Between Amlodipine Dosage Administered (mg/kg)" and Serum Testosterone Level After Treatment (ng/dL)"

Correlations

		Amlodipine_Dosage_mg_kg	Post_Treatment_Testosterone_ng_dL
Amlodipine_Dosage_mg_kg	Pearson Correlation	1	-.082
	Sig. (2-tailed)		.570
	N	50	50
Post_Treatment_Testosterone_ng_dL	Pearson Correlation	-.082	1
	Sig. (2-tailed)	.570	
	N	50	50

The correlation coefficient is -0.082, indicating a very weak negative correlation between Amlodipine Dosage and Post-Treatment Testosterone Levels. This means that as the dosage of amlodipine increases, testosterone levels tend to decrease slightly, but the relationship is extremely weak. The p-value is 0.570, which is greater than 0.05, indicating that the correlation is not statistically significant. This means there is no strong evidence to suggest that amlodipine dosage has a meaningful effect on testosterone levels.

DISCUSSION OF THE RESULTS

This study aimed to determine the levels of serum testosterone in animal models and the effect of the amlodipine on testosterone levels in the same. There was also no statistically significant difference ($p = 0.068$) in testosterone levels between baseline and after treatment with amlodipine. Furthermore, amlodipine dosage did not have less than modest effect on testosterone ($p = 0.732$) and did not correlate modestly and significantly with testosterone after therapy ($r = -0.082$, $p = 0.570$). These data imply that amlodipine does not significantly affect testosterone levels of the models being studied.

The effects of calcium channel blockers (CCBs) on reproductive hormones have been reported to be either positive or negative in past studies. For example, the administration of amlodipine in Sprague Dawley rats as per Latif et al. (2008) leads to the reduction of testosterone levels and testicular regression. But our findings are contrary to this as the different doses of amlodipine did not affect testosterone levels.

As reported by Onwuka et al. (2016) also, amlodipine decreases testosterone in rats, and this also supports the possibility that CCBs have a negative effect on reproductive function. But instead of dose dependent effects, we did not see any decreases of testosterone at higher dosages.

Additionally, Jamil et al. (2020) also reported that amlodipine treatment was accompanied by an increase in follicle stimulating hormone (FSH) and a decrease in testosterone, which may imply disturbance in reproductive hormone balance. While half of the test animals in our study behaved differently than the control animals, there were no corresponding hormonal changes, and other variables besides testosterone may influence reproductive behavior.

AlShebli (2017) also conducted a clinical investigation with which he demonstrates that male hypertension patients given amlodipine has lower serum testosterone levels. It was found in this study that the older patients are more severely suppressed in a dose dependent manner. We could not repeat these findings because the experimental conditions varied, the species difference or the duration of drug administration.

CONCLUSION

The aim of this study was to evaluate the effects of serum testosterone levels using an animal model after administration of amlodipine. The findings demonstrate that amlodipine treatment had no effect

on testosterone levels before and after therapy. There was also a weak, statistically insignificant relationship between the dosage of amlodipine and testosterone levels and the changes in drug dosage did not lead to significant changes in testosterone concentrations.

This contradicts earlier research that alleged that calcium channel blockers like amlodipine may prevent the testosterone generating by blocking calcium dependent enzymatic activity in Leydig cells. While half of the rats displayed changes in their reproductive behavior, these changes were not accompanied by changes in hormone levels detectable enough to suggest that the changes were due to altered hormone levels.

One explanation for the inconsistency between this study and work done previously is that species differences, amount of time the drug is administered, and possible compensatory biological mechanisms that would decrease the drug's effect on testosterone level. The study was also quite brief by comparison, and it may not have been able to pick-up long-term hormonal effects. However, these results do not preclude the possibility that amlodipine may adversely affect male reproductive health, as previous clinical reports have suggested that amlodipine suppresses testosterone in human beings. Further exploration is needed to determine whether amlodipine significantly affects male reproductive hormones, such as more extensive animal experiments and clinical trials. It is important to understand the consequences of sexual dysfunction and hormone imbalances on the hypertension patients' commitment to therapy, since sexual dysfunction and hormone imbalances can have an impact on hypertension patients' commitment to therapy.

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Questionnaire for Data Collection
Title: *Effects of Amlodipine on Serum Testosterone – Animal Study* Setting: Tertiary Care Hospital, Quetta
Researcher Name: ----------
Date of Data Collection: _____

Section 1: Animal Identification and General Information
1. Animal ID: _____
2. Species: ☐ Rat ☐ Rabbit ☐ Other: _____
3. Age (in months): _____
4. Weight (in grams/kg): _____
5. Sex: ☐ Male

Section 2: Group Classification and Drug Administration
6. Group Allocation:

☐ Control Group (No amlodipine)

☐ Low-Dose Group

☐ Medium-Dose Group

☐ High-Dose Group

7. Amlodipine Dosage Administered (mg/kg): _____
8. Duration of Treatment:

☐ 4 weeks ☐ 6 weeks

9. Route of Administration:

☐ Oral ☐ Intravenous

Section 3: Hormonal and Biochemical Analysis
10. Baseline Serum Testosterone Level (Before Treatment) (ng/dL): _____
11. Serum Testosterone Level After Treatment (ng/dL): _____
12. Percentage Change in Testosterone Level: _____

Section 4: Physiological and Behavioral Observations
13. Has there been any change in body weight during the study?

☐ Increased ☐ Decreased ☐ No change

14. Has there been any change in reproductive behavior?

☐ Yes ☐ No

15. If yes, what type of behavioral changes were observed? (Check all that apply)

☐ Reduced mating attempts

☐ Decreased aggression

☐ Lethargy or reduced activity

☐ Other: _____

16. Have there been any significant physical changes? (Check all that apply)

☐ Decreased muscle mass

☐ Increased fat accumulation

☐ Loss of fur/hair thinning

☐ No significant changes