

# Effect of Amlodipine and Captopril on Reproductive Capabilities of Male Laboratory Mice

Salma Saeed Abbas\*

Department of Biology, College of Education for Pure Science, University of Basra, IRAQ.

## ABSTRACT

**Background:** Hypertension is well confirmed as a major risk factor for cardiovascular disease. Researches in the treatment of hypertension has produced a multitude of drug classes with different efficacy profiles, which including  $\beta$ -blockers, diuretics, ACE inhibitors, angiotensin receptor blockers and calcium channel blockers. This study aimed to investigate the effects of captopril and amlodipine on some of reproductive abilities. **Materials and Methods:** The laboratory mice were 10-12 weeks and 23-25 g divided into three groups, six mice of each one. Group A served as control group; injected with 0.1 ml of normal saline, group B; injected with 0.1 ml of Captopril with 0.2 mg / kg, group C injected with 0.1 ml of amlodipine 0.08 mg / kg. **Results:** Decrease in sperm count and testis weights and testosterone hormone of Amlodipine treated mice. While the numbers of sperms and testis weight of captopril treated mice were comparable to that of control

groups. But decrease normal sperms of captopril and Amlodipine treated mice comparable to that of control group. **Conclusion:** These results suggest that the treatment with amlodipine causes a significant testicular weight regression and reduction in sperm count.

**Key words:** Mice, Amlodipin, Captopril, Sex hormones, Sperms.

## Correspondence

Dr. Salma Saeed Abbas,

Department of Biology, College of Education for Pure Science, University of Basra, IRAQ.

Phone no: +009647709064097

Email: salmaabbas300@gmail.com

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## INTRODUCTION

The blood is carried around the body in tubes called blood vessels. The pumping of the heart keeps blood moving through the blood vessels. Blood pressure is the force of blood pushing against blood vessel walls. It is measured in millimeters of mercury (mm Hg). High blood pressure (HBP) means the pressure in your arteries is higher than it should be. Another name for high blood pressure is hypertension.<sup>1</sup> Many blood pressure medications, known as antihypertensive, are available by prescription to lower high blood pressure (HBP or hypertension).<sup>2</sup> As widely accepted, sperm counts may vary among different ejaculates according to several pathological conditions, lifestyle and exposure to pollutants.<sup>3</sup> Several "acute" pharmacological treatments, as antibiotics, could cause subclinical and temporary reduction of male fertility; conversely, long-term medical treatment may severely affect male fertility, although this effect could be considered transient in most of the cases. Thus, nowadays, several long-term pharmacological treatments may represent a clinical challenge. To the best of our knowledge, association between several kind of antihypertensive drugs and reduction of male fertility has been showed in the mouse model.<sup>4,5</sup>

In particular, beta blockers and Calcium-channel Blockers (CCBs) seem to play a detrimental role on male fertility, causing in several cases azoospermia and/or oligozoospermia. In this regard, it was already demonstrated in the mouse model that CCBs, like amlodipine, can cause a reduction of testosterone, luteinizing hormone (LH) and follicular stimulating hormone (FSH), leading to affect spermatogenesis and sperm parameters.<sup>6,7</sup> Calcium ions have a ubiquitous presence in somatic and germ cells.<sup>8,9</sup> Calcium ions are, therefore, directly involved in the regulation of the following key processes that regulate or determine male fertility: blood testicular barrier,<sup>10,11</sup> testosterone synthesis by Leydig cells,<sup>12</sup> hormonal regulation of Sertoli cells function,<sup>13</sup> secretion of products by Sertoli cells,<sup>7,8</sup> capacitation of sperm cells,<sup>14</sup> sperm motility,<sup>15</sup> spermatogenesis, penetration of oocytes by sperm cells, prevention of polyspermy and gene expression.<sup>8,9</sup> However, increased intrasperm concentration of calcium ions was determined to correlate negatively with sperm viability.<sup>16</sup> Captopril is a commonly used antihypertensive drug that acts by inhibiting the activity of angiotensin-converting enzyme ACE.<sup>17</sup> Moreover, a number of *in vitro* studies have confirmed the activity of ACE in semen. Captopril at 100 nmol L<sup>-1</sup> inhibits the ACE activity in cell culture of the rat cauda epididymis, which confirms the presence of ACE in these cells.<sup>18</sup> Another *in vitro* study showed that ACE was localised in the periacrosomal area of ejaculated spermatozoa.<sup>19</sup>

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## MATERIALS AND METHODS

### The laboratory animals

In the current study, the experimental animals were *Mus musculus* L. from Balb / C, which prepared from the Department of Pharmaceutical Control of Baghdad Governorate and raised in the Animal House of the Department of Life Sciences, College of Education for Pure Sciences, Basrah University under similar conditions, 20-25°C and a 12 hr fixed lighting system 12 hr light.<sup>20</sup> The mice were placed in plastic cages of standard size (30 x 12 x 11 cm). The floor of the cages was sprayed with wood shavings, which are replaced weekly and the animals were fed in a specific diet.<sup>21</sup> The laboratory mice were 10-12 weeks and 23-25 g divided into three groups, six mice of each one. Group A served as control group; injected with 0.1 ml of normal saline, group B; injected with 0.1 ml of Captopril with a concentration of 0.2 mg / kg, group C injected with 0.1 ml of amlodipine 0.08 mg / kg concentration.

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## Serum preparation

The blood serum was prepared by withdrawing the blood directly from the heart at Day 31. After treating the animals with chloroform, the tubes were placed in the centrifuge at a speed of 3,500 cycles per minute.

## Physiological tests

**Test of Captopril and amlodipine in the calculation of sperm count in laboratory mice:** The method of vega *et al.*<sup>22</sup> was used in the calculation of the sperms. The right epididymis is taken from the mouse and cut by a blade and placed in a petri dish with Normal Saline.

**Test of captopril and amlodipine effect on malformation of sperms:** The Wyrobek and Bruce method,<sup>23</sup> was used, where the sperm were taken from the left epididymis of the mouse and placed in a Petri dish of phosphate buffer saline and the sperms classified to normal sperm, malformed sperms.

**Test of amlodipine and captopril effect on testis weight:** According to weight of testis after fixated it with 10% formalin and then preserve it with 70% ethanol.

**Effect of amlodipine and captopril in testosterone and luteinizing hormone:** Method of kosasa<sup>24</sup> was used in this test to estimate LH and also by method of kick lighter and Norman.<sup>25</sup>

## Statistical Analyses

The statistical analysis are performed using SPSS version 20 with  $P < 0.05$  at a significant. Data are expressed according to Mann-Whitney Test, Kruskal-Wallis Test and multivariate ANOVA.

## RESULTS

### Effect of Captopril Amlodipine in the rate of sperms count and testis weight of the laboratory mice

The results of the current study, as shown in Table 1, showed decrease in number of sperm and testis weights of Amlodipine treated mice. While the numbers of sperms and testis weight of captopril treated mice were comparable to that of control groups.

### Effect of Captopril and Amlodipine in the induction of laboratory mice sperms abnormalities

The results of the statistical analysis shown in Table 2 and Figures 1-9 showed a significant decrease in the number of normal sperm and a significant increase in sperm abnormalities for both drugs and at a level of probability ( $P < 0.05$ ) compared to control group.

### Effect of Captopril and Amlodipine in the level of testosterone hormone and the Luteinizing hormone.

The current study indicated in Table 3 showed a significant decrease in the level of testosterone for mice treated with Amlodipine and a significant increase in the level of such hormone of mice that treated with Captopril compared with the control group at a level of probability ( $p < 0.05$ ) compared to control group. The statistical analysis did not give any significant differences in the level of the luteinizing hormone of the groups (Captopril and Amlodipine).

## DISCUSSION

Amlodipine is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina.<sup>1</sup> Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart

**Table 1: Effect of amlodipine and captopril in sperms count and testis weight of the laboratory mice.**

Treated groups	Sperm count mm <sup>3</sup> x10 <sup>6</sup>	Testis weight (g)
Control	8.01+0.82	0.0635+0.0072
Captopril(0.2mg/kg)	8.82+0.94	0.0644+0.0055
amlodipine(0.08mg/kg)	5.75+0.12	0.0496+0.0079

**Table 2: Effect of Captopril and Amlodipine in the induction of laboratory mice sperm abnormalities.**

Treated groups	Normal sperms (%)	Distorted sperms (%)
Control	75+1.75	25+1.57
Captopril (0.2 mg/kg)	56+3.3*	44+3.38*
Amlodipine (0.08 mg/kg)	64+0.178*	36+2.7*

There were significant differences at the probability level ( $P < 0.05$ ) compared with control group.

**Table 3: Effect of amlodipine and captopril in testosterone level and luteinizing hormone.**

Treatment	Testosterone H (ng/ml)	Luteinizing H IU/l
Control	4.41+1.75	6.77+1.0
Captopril(0.2mg/kg)	5.44+0.4 *	6.25+3.38
Amlodipine(0.08mg/kg)	2.45+0.6 *	6.39+0.3

Presence of significant differences ( $p \leq 0.05$ ) in the level of testosterone hormone compared with the control group.



**Figure 1:** Normal sperm.



**Figure 2:** Abnormal head of sperm



**Figure 3:** Abnormal sperm with distorted hook.



**Figure 7:** Abnormal sperm appears the tail splits



**Figure 4:** Abnormal sperm with spherical head



**Figure 8:** Abnormal sperm appears ball in middle segment



**Figure 5:** Abnormal sperm showing the coalescence of the medial segment



**Figure 9:** Abnormal sperm with distortion in middle part and tail



**Figure 6:** Abnormal sperm showing the curvature of medial segment

muscle, Amlodipine does also act as functional inhibitor of acid sphingomyelinase.<sup>2</sup> While the Captopril is a commonly used antihypertensive drug.<sup>26</sup> That acts by inhibiting the activity of angiotensin-converting enzyme (ACE).<sup>27</sup> A zinc-containing metalloproteinase enzyme catalysing the conversion of angiotensin I into angiotensin II (a potent vasoconstrictor).<sup>28</sup> Decreased angiotensin II synthesis reduces the activity of the renin angiotensin aldosterone system and hence the blood pressure as a result of vasodilation, which occurs in response to increased production of bradykinin and vasodilatory prostaglandins and decreased water and salt retention.<sup>29</sup>

Results of the current study showed a decrease in the weights of testis and the preparation of sperm and the level of hormone lipid testicular in the group of animals treated with amlodipine compared to the control group and this result is identical to the study of,<sup>30</sup> who found that the

drug Amlodipine cause a Decreased rats' testicular weights and were also identical to Oluwaseun's study,<sup>31</sup> as there was a decrease in live sperm count for animals treated with amlodipine, while Captopril showed no effect on testis and sperms count.

The reason for the decrease in the level of testosterone hormone, normal sperm and the size and weight of the testicles may be attributed to the action of calcium channel blockers in the hypothalamic axis and changes in the hypothalamus released from the hypothalamus or the induced hormones of the pituitary gland which agreed with the results obtained by,<sup>32</sup> if there is a decrease in the hormones that feed the fetus by the effect of calcium channel blockers, the decrease may be caused by stopping the process of the emergence of sperm in the testis due to the absorption of calcium ion in the testis. As a result, the sperm density decreases and its effect is blocked Calcium Suresh. Stopping the process of sperm formation supports the reduction in the weight of the testicle by the effect of the Cretaceous drug. At this stage, the conclusion is that the amlodipine causes a significant drop in testicular weight, sperm count and serum testosterone levels. While the Captopril effect on semen quality such as deformities but not quantity.

## CONCLUSION

In conclusions, this study has shown that the amlodipine causes a significant drop in testicular weight, sperm count and serum testosterone levels. While the Captopril effect on semen quality such as deformities but not quantity.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest

## ABBREVIATIONS

**ACE:** Angiotensin-converting enzyme; **CCBs:** Calcium-channel Blockers; **FSH:** Follicular stimulating hormone; **HBP:** Hypertension; **LH:** Luteinizing hormone.

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