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Sex hormones and sperm parameters of male white rats treated with amlodipine

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Abstract---The study aimed to investigate the effect of amlodipine on Sex hormones and sperm parameters in white male rats. The study was conducted in the animal house of the College of Pharmacy/University of Karbala, And on October 31, 2021 until January 30, 2022. Twenty (20) adult white rats, a three-month-old male, with weights ranging from (300-200) g. Male rats were randomly divided into two main groups, the first group was a control group that included (10) rats, It was dosed orally and daily with physiological saline solution (NaCl 0.09%), As for the second group, it was dose it orally the amlodipine at a concentration of (10 mg/kg) for 60 day. study showed a significant decrease ($P \leq 0.01$) in the sperm concentration in the caudal epididymis And in the percentage of live sperm in the caudal epididymis and sperm motility as well as in the viability of sperm, Also, there was a significant increase ($P \leq 0.01$) in the percentage of upnormal sperms in the caudal epididymis when compared to the control group. The results of the current study showed a significant decrease ($P \leq 0.01$) ($P \leq 0.05$) for (Follicle-Stimulating hormone (FSH) and Luteinizing hormone (LH) and Testosterone hormone).

Keywords---Amlodipine, Rats, sperm motility, sperm viability, Testosterone.

Introduction

Infertility and impotence are among the biggest of these problems in couples, as it has been recorded in the past few years about 30% of cases of infertility in couples, which are due to male factors (Isidori et al., 2006), There are many reasons that can interfere with the process of sperm synthesis and reduce their quality and production, the most important of which is drug treatment,

Chemotherapy, air pollution, toxins and lack of vitamins taken. Which can cause harmful effects on the process of synthesis and natural production of sperm (Mosher & Pratt, 1991). Calcium ions are found in germ cells and contribute mainly to the main processes that regulate or determine male fertility, including regulation of the blood-testis barrier, Testosterone synthesis by Leydig cells, Regulating the function of secretory Sertoli cells, spermatogenesis, sperm motility and endosome interactions, spermatogenesis, And the penetration of the ovum by sperm and others. Amlodipine is a commonly used drug. It is a vasodilator drug used to treat patients suffering from high blood pressure and angina pectoris, It belongs to the family of calcium channel blockers, Which works to prevent the flow of calcium ions in the blood vessels smoothly in the muscle cells and reduce their blood pressure by reducing the resistance of peripheral blood vessels, Amlodipine is a long-acting calcium channel blocker (dihydropyridine class) used as an antihypertensive and in the treatment of angina pectoris, Like other calcium channel blockers. The current study aims to know the effects of amlodipine to know the extent of its effect on male sex hormones and sperms by knowing:

- Estimation of the level of testosterone hormone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH).
- Studying Sperm parameters, which included estimating the number of sperm counts, and the percentage of sperm motility, sperm viability, distorted sperm.

Materials and Methods

In this study, (20) white male rats were used, which were in a healthy condition, sexually mature at the age of three months, with weights ranging between (200-300 g). The laboratory animals were brought from the College of Pharmacy / University of Karbala, and placed in the animal house of the College of Pharmacy / University of Karbala in plastic cages. And they were taken care of under laboratory conditions of good lighting (11 hours of light / 13 hours of darkness), good and regular ventilation and appropriate temperatures ranging between (27-23) degrees Celsius, Appropriate bedding was placed in the cages and cleaned twice a week. As for feeding the animals, they were given water and food suitable for growth, which contain a high percentage of protein Throughout the experiment, Rats were divided into two groups: **The first group:** the control group, which included (10) adult male rats, as these animals were dosed (1ml) Normal Saline for 60 consecutive days, **The second group:** The group that was dosed orally with the drug Amlodipine at a dose of (10 mg/kg) for (60) days, which included (10) adult male rats, and the dose was done using a sterile oral gavage for (60) days. After 24 hours of the last day of dosing, all animals were sacrificed by anesthetizing the animals using anesthetic (chloroform) by placing a cotton-tipped cotton container on the anesthetic in a large box containing the rat to be partially anesthetized by breathing. To ensure that the heart remains working hard enough when blood is drawn directly from the heart with a Heart Puncture, By means of 5 ml wine medical syringes, then the blood is placed in dry, sterile tubes, free of anticoagulant, and the blood is left for an hour to take sufficient time to clot, after which the blood is transfused, To a centrifuge to separate it at a speed of 5000 rpm for 5 minutes to obtain the serum, which was placed in special tubes (Appendrof tube) and kept in the refrigerator at a temperature of -20°C until hormones were measured. The concentrations of hormones in the blood serum

were measured using a measuring kit (KIT) by an ELISA reader manufactured by Monobind- Inc., of American origin, By using an enzyme-linked immunosorbent assay (ELISA) The levels of hormones in the serum were estimated, and the absorbance was read at a wavelength of 450 nm, as the concentrations of the following hormones were measured, (Testosterone hormone (T)- follicle stimulating hormone (FSH)-luteinizing hormone (LH). After postmortem of the rats and extracting the testes and epididymis, the sperms were collected from the left tail of the epididymis, where the sperms were placed in (10ml) of physiological solution in a Petri dish, after that we perform the sperm count by placing a drop on the glass slide and examining it under a light microscope (Elbetieha *et al.*,2009). **Sperms Motility:** A drop of semen is placed on a warm slide at a temperature of 37 degrees Celsius and examined under a microscope with a power of X400. The active movement of sperm is expressed by the following equation: (percentage of motility % = number of motility sperms / total number of sperms) x100. according to the method of (Silverbege and Turner ,2001) **Sperm concentration :** The sperm concentration was calculated according to the method (Hinting , 1989). **Sperm Viability Percent:** Mix a drop of sperm with a drop of eosin and necrocin dye. After that, the sperm, eosin and necrocin are mixed using a fixed angle slide. Then a part of the mixture is taken at the tip of the second slide as well as the third slide, to make a light smear. Leave the third slide to dry at room temperature, then check. The slides, where the dead sperm take the dye, while the live sperm are not dyed, and the percentage of the live sperm is calculated. (Number of live sperm / total number of sperm) x 100. (Hafez,1987). **Abnormal Sperm Percent:** The sperm was examined and the normal appearance was distinguished from the distorted by the head or tail or both to calculate the abnormal sperm. The same slide was used that was used to calculate the dead and live sperm, and to determine the abnormal appearance of the sperm, the following equation is calculated: (Deformation% = Abnormal number of sperms / Total number of sperms X 100), (Dale and Edler, 1997), (Damascono *et al.*, 2008).

Results and Discussion

The results of the statistical analysis of the current study are shown in Table (3-4) There are significant differences in concentrations (Testosterone hormone (T) - follicle stimulating hormone (FSH)-luteinizing hormone (LH), Where a significant decrease ($P \leq 0.01$) was observed for each of the luteinizing hormone(LH) and follicle stimulating hormone (FSH), And a significant decrease at ($P \leq 0.05$) for Testosterone hormone. For the group of animals treated with amlodipine (10 mg/kg) for two months And on a daily basis when compared to the control group.

Table (3-4) study the effect of amlodipine (10mg/kg) on hormone levels in male rats dosed orally for (60) days compared to the control group

Averages $\pm$ Standard Error			hormones groups
Testosterone (mIU/ml)	LH (mIU/ml)	FSH (mIU/ml)	
0.68 $\pm$ 0.03	3.17 $\pm$ 0.23	14.93 $\pm$ 1.44	treatment group
2.06 $\pm$ 0.49	6.92 $\pm$ 0.62	53.66 $\pm$ 6.47	control group

*	**	**	Morale
** (P≤0.01)	* (P≤0.05)		

The results of the current study showed a significant decrease in the level of the hormone (FSH, LH, Testosterone), and this is similar to what was reached (Alshebli 2013) Which conducted a study in Al-Sadr Teaching Hospital on males who use amlodipine , Also, the results of our study agree with (Onwuka, F et al., 2010) who conducted an experiment on white male rats using amlodipine for 6 weeks. . The results also agreed with what was obtained (Rabia Latif et al., 2008), which recorded a decrease in testosterone during its study on rats dosed with amlodipine for 50 days. The reason for the decrease in these hormones is due to calcium channel blockers such as amlodipine, which caused blockage of the calcium channel and inhibiting the entry of calcium into the gonadotrophs. Which leads to the suppression of the expression of the genes LHB and FSHB, which leads to the inhibition of the synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and the reduction of testosterone, The release of GNRH from the pituitary glands is calcium dependent, so the induction/repression of the LHB and FSHB genes depends on the calcium influx (Haisenleder et al., 2003). Calcium plays an important role in the secretion of the hormone GnRH directed to stimulate the hormones LH, FSH (Taranta et al., 1997). The hormonal stimulation of testicular development and testosterone secretion involves the influx of calcium, possibly through calcium channels in Lydeck cell membranes (Manna et al, 1999). A decrease in the level of testosterone in the blood due to the drug amlodipine can either be a direct effect of the drug on the Lydc cell or an indirect effect through the disturbance of the hormonal environment in the pituitary gland axis, which causes inhibition of the release of the hormone LH and FSH and reduce its levels. Thus reducing the testosterone hormone (Weinbauer *et al.*,1991). also mentioned (Benoff S.1998) that amlodipine is one of the drugs that prevents the flow of calcium ions through calcium channels, which have a vital role in the secretion of sex hormones from the pituitary glands and testicles, Thus, the activity of these hormones decreases . The presence of calcium channel blockers reduces the release of GNRH from hypothalamic neurons, which leads to a decrease in the secretion of LH and FSH and thus a decrease in the level of testosterone (Bourguignon *et al.*,1987). An in vivo study showed that amlodipine is able to inhibit gonadotropin secretion, as well as decrease the release of LH and FSH hormones in response to GNRH inhibition, and as a result, the level of testosterone also decreased (Barbarino et al, 1982).

And The results of the statistical analysis of the current study as shown in Table (4-4) showed significant differences ($P \leq 0.01$), it was clear that there was a significant decrease in the rate of sperm concentration in the tail of the epididymis and a significant decrease in the percentage of live sperm in the tail of the epididymis and percentage of motile sperm, As well as in the degree of sperm activity, for the group dosed with amlodipine at a concentration of (10 mg/kg) for two months and on a daily basis compared to the control group. Also, the treatment of rats with amlodipine resulted in a significant increase ($P \leq 0.01$) in the percentage of homozygous sperms in the caudal epididymis when compared to the group that was not given the drug.

Table (4-4) study the effect of amlodipine (10mg/Kg) on sperm parameters in male rats dosed orally for (60) days compared to the control group

Averages $\pm$ Standard Error					Sperm criteria
Sperm activity%	sperm motility%	abnormal sperm%	Sperm Viability %	Sperm concentration	
0.20 $\pm$ 0.02	1.40 $\pm$ 0.98	85.6 $\pm$ 1.96	19.2 $\pm$ 1.48	20.4 $\pm$ 6.5	Treatment Group
65.6 $\pm$ 6.62	89.6 $\pm$ 0.75	9.0 $\pm$ 0.71	89.6 $\pm$ 2.77	78.8 $\pm$ 2.08	control Group
**	**	**	**	**	

** ($P \leq 0.01$)

The results of this study agreed with (AVDATEK, F. 2016) and (Almeida et al, 2000), Also, our current study agreed with the findings, (ds.Akinlolu et al., 2013). In his experiment, administration of amlodipine caused a detrimental effect on reproductive function and on the number of mature sperm . This effect may be attributed to the decrease in the secretion of FSH and LH hormones from the anterior lobe of the pituitary gland, which leads to the inhibition of testosterone production, which affects the process of spermatogenesis, and consequently, the decrease in the concentration of sperm inside the seminiferous tubules and the fertility of animals. The production of naturally mature sperm is the basis of male fertility, (Muthulakshmi *et al.*,2013). The production of sperm and testosterone in the testicles is mainly regulated by the hormones Follicle Stimulate Hormone (FSH) and Lutein Hormone (LH) , Which are released from the pituitary gland, which are the main regulators of the process of spermatogenesis (Guyton and Hall, 2001). Low level of gonadotropin nutrients may have inhibited the initiation of spermatogenesis in treated rats, Because inhibition of the anatomical nutrients prevents the signal responsible for the initiation and completion of the process of spermatogenesis during the normal maturation of the developing sperm (Neuman et al., 2002). Also, initiating the process of sperm formation and maintaining it quantitatively and qualitatively naturally requires the presence of sufficient levels of the hormones that nourish the gonads and the hormone Testosterone, Inadequate levels of these hormones are usually associated with severe abnormalities in spermatogenesis and thus may lead to oligospermia or asthenia. Moreover, the function of the accessory gonads also depends on the presence of adequate levels of Testosterone in the circulatory system. (Almenara *et al.*, 2000; Nishino *et al.*, 2004), Which works on the development of spermatozoa and differentiation of spermatogonia and prospermia and also contributes to the generation of FSH receptors in Sertoli cells (Guyton and Hall, 2001). Androgen also plays an important role in the final stages of sperm development (Bustos-Obregon et al., 2006) , As it stimulates the transformation of round spermatids into elongated ones during the spermatogenesis cycle, and androgen deficiency disturbs the process of sperm liberation (Saito et al, 2000), By changing the spermatogenic contact points with Sertoli cells which leads to premature detachment of the round precursors from Sertoli cells and the seminiferous epithelium (Beardsley and O'Donnell, 2003). And several researchers reported that inhibiting the effectiveness of hormones feeding the hormones is responsible for the decline in fertility in Rate. It was found that amlodipine acts directly or indirectly on the secretory function of the pituitary gland, causing a decrease in

the concentration of sperm in the epididymis through a decrease in the secretion of androgens (Bazrafkan *et al.*, 2010). (Dominic and Padjama 2013), (Juneja and Gupta, 1990), (Chalob *et al.*, 2013), who found in their experiments on animals that amlodipine caused a decrease in sperm motility and an increase in the number of dead sperm, and this It also agrees with the findings of our current study. The fact that amlodipine caused a significant decrease in sperm motility indicates that this drug is able to penetrate the blood barrier in the testicle with a resulting change in the microenvironment of the seminiferous tubes, where it has been reported that the decrease in sperm motility caused by chemical factors was due to its ability to permeate the blood barrier of the testicle (Baldessarini RJ. 1980), and thus create a different environment in the inner part of the wall of the seminiferous tubules than the outer part (Bloom *et al.*, 1975). The decrease in sperm vitality, as well as the moral increase in the percentage of abnormally shaped sperm induced after treatment of rats with amlodipine, may be due to the drug's ability to interfere with the sperm-generating processes in the seminal tubes and the functions of the epididymis, which leads to a change in sperm formation (Bowman And, Rand.1985), (Wiliam *et al.*, .2000), and this is similar to the result of the experiment (Oyededeji *et al.*, 2013) in isolated rats treated with tetracyclic steroid.

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