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Article

Caffeine Induced Histopathological and Hormonal Alterations in Adult Rabbits

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Abstract: In recent years, the consumption of caffeine has significantly increased worldwide. However, the potential negative effects of caffeine on the reproductive system have raised concerns among researchers and health professionals. The present study aims to investigate the effects of caffeine on the histology of the liver and testes of male rabbits and caffeine relation to reproductive hormones. Twenty-one adult male rabbits Florida White were divided into three groups. The Control Group was given 2ml of normal saline, while the second (G1) was given 2ml caffeine orally at a dosage of 2 mg/ml, the third group (G2) was given 2ml caffeine orally at a dosage of 6 mg/ml, for month. At the end of the experiment, liver and testes were obtained and stained with H & E stains. Blood samples were also collected to study; thyroid stimulating hormone (TSH), follicular stimulating hormone (FSH), and luteinizing hormone (LH). Histological results revealed that caffeine cause disorganized germinal epithelium of seminiferous tubules and marked vacuolations with a pronuclear of sperm. Moreover, in the liver there were necrosis of hepatocytes, fatty degeneration. There was significant decrease in the concentrations of FSH, LH, and TSH levels in both (G1) and (G2) compared with the control group. In conclusion, caffeine had an adverse effect on liver and testes characterized by tissue degeneration, cellular necrosis, and structural disruption evidenced by restoring the normal state when the animals were banned from further caffeine treatment.

Keywords: caffeine, histology, hormones, testes, liver.

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1. Introduction

In recent years, the consumption of caffeine has significantly increased worldwide, [1]. Caffeine, a natural stimulant found in various beverages and food items, as well as in some therapeutic products such as cold remedies and allergy drugs, is known for its ability to enhance alertness and reduce fatigue. However, the potential negative effects of caffeine on the reproductive system have raised concerns among researchers and health professionals alike [2][3]. Although many existing researches have addressed the effects of caffeine on female fertility, conception, fecundity, and birth weight as well as fetal health, it is very important to consider the effects of caffeine on male fertility. It is crucial to understand the anatomy and histology of the male reproductive system in order to comprehend the potential effects of caffeine on its functioning. The most significant organs within the male reproductive system are the testicles. Additionally, the process

known as spermatogenesis involves the production of sperm by the testes. It is therefore important to understand how does caffeine affects the structural integrity of the testes. The testes are the male reproductive glands of the testes. According to Klaus *et al.*, (2011) [4], the testes, like the ovaries, are integral components of both the reproductive and endocrine systems. The primary functions of the testes include the production of sperm (Spermatogenesis) and the production of androgens, primarily testosterone. Both testicular functions are influenced by the gonadotropic hormones produced by the anterior pituitary. Luteinizing hormone (LH) results in testosterone release [5]. Simoni *et al.*, (2017) [6], suggest that the presence of both Testosterone and Follicle-Stimulating Hormone (FSH) is necessary to support spermatogenesis. Almost all healthy male vertebrates have at least two testicles. They are typically of comparable size, although that may vary depending on the species. The tunica albuginea structurally covers the entire testis. Under the tough tunica albuginea, the testes contain very fine coiled tubes called seminiferous tubules, within which spermatogenesis takes place. The rabbit testis was a tubular gland found within the scrotum that was enclosed by a strong connective tissue capsule. This connective tissue capsule produces several septa and divides the testicular parenchyma into multiple lobules. The seminiferous tubules were found in each lobule, while the Leydig cells located in the heavily vascularized interstitial spaces. The seminiferous tubules were made up of a multilayered or stratified epithelium that included spermatogenic cells at various stages of development. Sertoli cells were detected in the seminiferous epithelium [7]. Caffeine consumption has negative effects on the testis and reproductive function [8]. Therefore, this study aimed to determine the histological effects of caffeine on the testes and liver of rabbits. This study also sheds light on which hormones may be affected by exposure to caffeine.

2. Materials and Methods

Ethical approve

According to the ethical code number UOK.VET.2024.105 from College of Veterinary Medicine, University of Karbala, Iraq, experimental animals and trials were allowed.

Animals

Experimental routine was authorized by the Veterinary Medicine College of Karbala University. Twenty-one adult male rabbits (Florida White) with ages between 9-10 months and weight about (1.8-2.3) kg were obtained from the house of laboratory animals in the Biotechnology Research Center of the college. The animals were healthy and free from any gross pathological lesions. The study was carried out for four consecutive weeks, during the 2023-2024 period.

Experiment design:

Twenty-one male rabbits were randomly divided into three equal groups (n=7). The Control Group was given 2ml distilled water. Group 1 was given 2ml caffeine orally at a dose of 2mg/ml. Group 2 was given 2ml caffeine orally at a dose of 6mg/ml. The dose of 2mg/kg was chosen according to previous study [9] with some modifications by using of higher dose 4mg/kg and lower dose 6mg/kg. The experiment was carried on for four consecutive weeks. Fresh feed and clean water were provided daily to the rabbits throughout the period of the experiment. The animals were maintained under the condition of natural light and dark cycles, (12 hours light, 12 hours dark), with recorded any clinical signs that appeared on the animals.

Blood sample collection:

Blood samples were collected from the animals; before the start of the experiment, using the ear vein, and at the end of the experiment, by cardiac puncture. A sterile needle with 5ml capacity was inserted into the heart and blood was collected. The blood was then put into a gel tube and left to clot. After that, blood was centrifuged to collect the serum. The serum was then analyzed to determine the effect of caffeine on the levels of thyroid stimulating hormone (TSH), follicular stimulating hormone (FSH), and luteinizing hormone (LH).

Animal sacrifice:

At the end of the experiment (after 4 weeks), the animals were sacrificed. Organ's testes and liver, were excised, and stored in 10% Formalin for further histological examination [10].

Tissues processing:

The testes and liver of rabbits were dissected and fixed in 10% neutral buffered formalin for 48-72 hrs. at room temperature. Then, tissues were dehydrated in graded ethanol series and embedded in paraffin wax. On rotary microtome device, the sections were cut at the thickness of 6μm, deparaffinized in xylene, hydrated in graded ethanol, and then exposed to staining with H & E stains for general observation of histological alterations.

3. Results and Discussion

In the examination of the microscopic section of the male rabbits; the Control group showed normal histological architecture of testicular and hepatic tissue, similar to the line with other studies in which they mentioned there are basic structures (seminiferous tubules, Leydig cells,) of testicular structure in addition to normal germinal epithelia of vital organs of aimed research involved the testes which are composed of tightly packed seminiferous tubules surrounded by a vascularized interstitial, and are enclosed in a tough fibrous capsule called the tunica albuginea. The liver showed the normal design architect of the hepatic structure (Central vein, Hepatocyte), and normal structure of the vital organs of the aimed research involving the liver which is composed of 8 segments that consist of 1,000 lobules (small lobes), each of these lobules is composed of hepatocytes, which have physiologically distinct apical and basolateral membranes, as notable in Figure (1) and (2). This is in line with other studies in which they mention basic structures [4],[11].

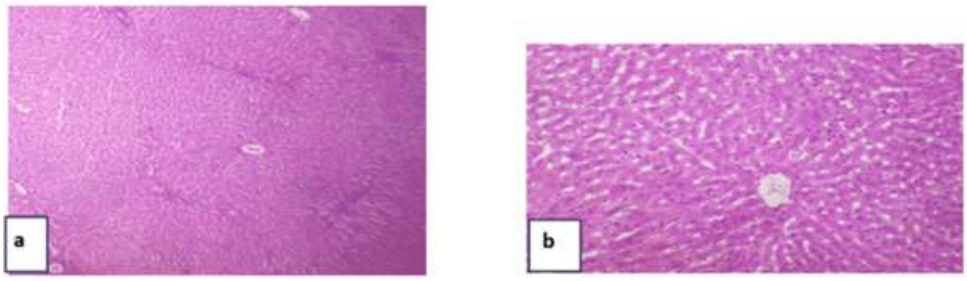

Figure 1. Micrograph of rabbit testes in control group: showed the basic normal structures (a) seminiferous tubules, (b)testicular tissue (Leydig cells); normal germinal epithelia (d), central luminal sperm (c). H&E, (a:10x and b:40x)

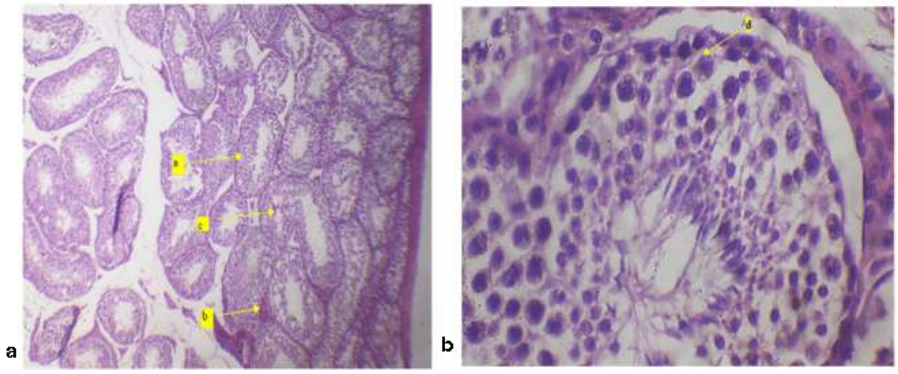

Figure 2. Micrograph of the rabbit liver in control group: shown the basic structures of the liver: central vein (light blue arrow), lobe (dark blue arrow) with normal hepatic cords (green arrow). H&E 40X, 4X.

However, Caffeine given to rabbits in the treated groups was seen to cause adverse effects on the normal structure of the testes. In group 1 rabbits that were treated with (2mg/ml) of caffeine, there was disorder in the seminiferous tubules, as it appears reduced in the size of the seminiferous tubules. Likewise, some tubules were containing clusters of degenerated germ cells, with presence of few sperms. As well, lysis of Leydig cells and blood vessel congestion were observed (Figure 3). These findings were agreed with previous researches [12][13][14]. Furthermore, it was reported that there was a decrease in the reproduction organs' weight, as well as its effect on the epithelial integrity and necrosis of the testes, with a significant decrease in germ cells [15][16][17].

Furthermore, in the liver of Group 1 that was received (2mg/ml) caffeine, there were noticed disorder in the its architecture and pyknotic also there is the presence of necrosis of hepatocytes with fatty degeneration, moderate hemorrhage in parenchymal hepatic tissue, and inflammatory cells infiltrations (Figure 4).

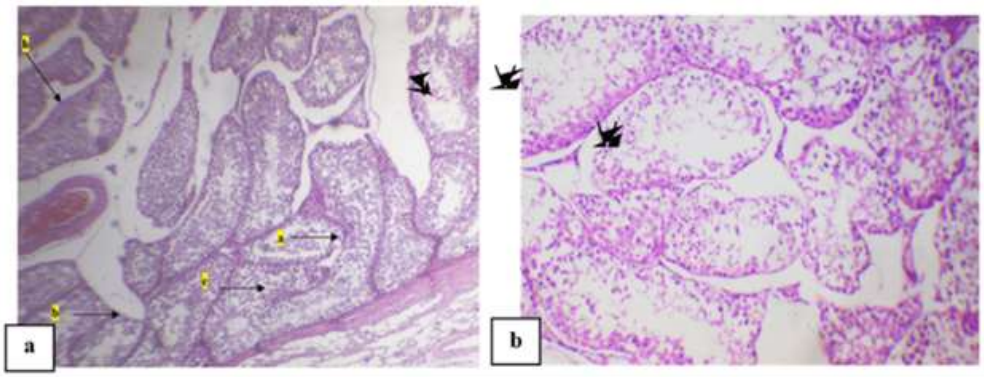

Figure 3. Micrograph of rabbit testicular tissue in group treated by (2mg/ml) of caffeine showed irregular appearance and reduced sizes of the seminiferous tubules (a, b). Some tubules contain clusters of degenerated germ cells, with few sperms (star), and blood vessel congestion. A:4x, b:10x, H&E.

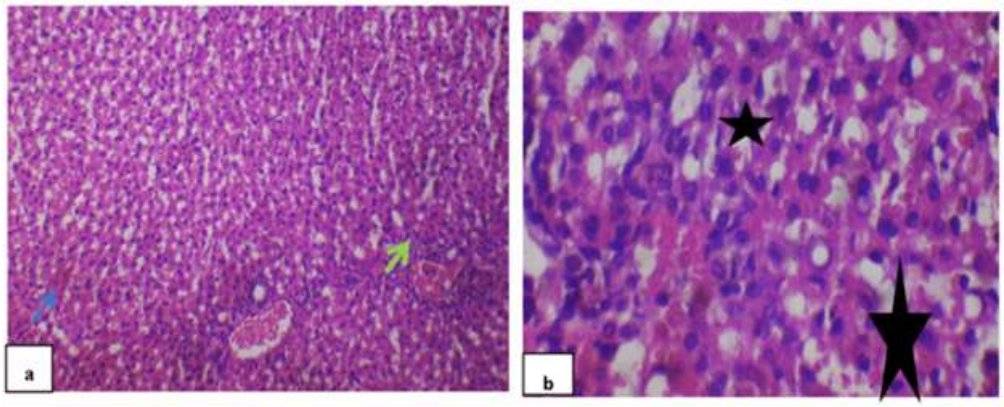

Figure 4. Micrograph of liver from examined rabbit in the group treated with (2mg/ml) of caffeine: shown disorder of liver architecture, necrosis of hepatocyte (red arrow) with fatty degeneration (star) and moderate hemorrhage in parenchymal tissue (blue arrow), inflammatory cells infiltrations (green arrow). a:10x, b:40x, H&E.

Caffeine was given to Group 2 that were treated with (6 mg/ml) caffeine, in the testes we noticed an irregularity in the size and shape of the seminiferous tubules. Some tubules were seen containing clusters of degenerating germ cells, with presences of few sperm. Also, damage to the germinal epithelium, and vacuolations. Moreover, there were

decrease in the number and size of Leydig cells, with blood vessel congestion, Figure (5). These findings are in accordance with previous studies [14], [16],[17].

In the liver of animals treated with (6 mg/ml) caffeine, there were noticed a disorder in the structure of the lobe liver and pyknotic, with the presence of necrosis of hepatocytes, hemorrhage in parenchymal hepatic tissue were also noticed. Correspondingly, congestion of blood vessels and inflammatory cell infiltrations were observed, with fatty degeneration of the liver, Figure (6). These results are similar toother research [17],[18].

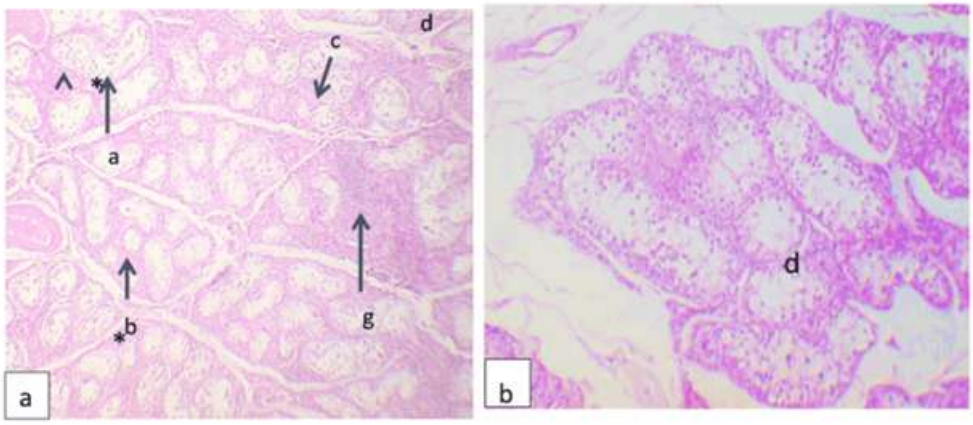

Figure 5. Micrograph of testicular tissue from examined rabbit in group treated (6mg/ml) of caffeine: shown irregular size and shape of seminiferous (a, b, c), Some tubules contain clusters of degenerating germ cells, with few sperm (d), decrease number and size of Leydig cells are observed with blood vessel congestion, a:4x, b:x40, H&E.

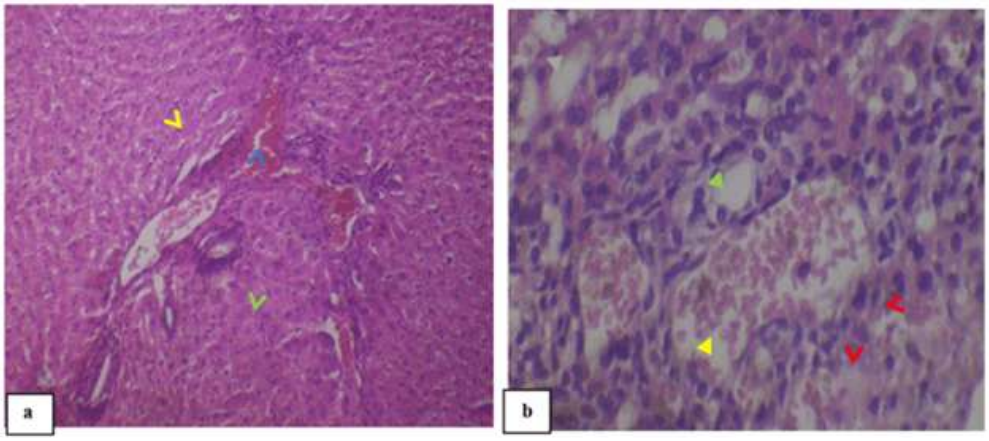

Figure 6. Micrograph of liver from examined rabbit in group treated (6mg/ml) of caffeine: shown the Disorder structure of the lobe liver with necrosis of hepatocyte (red arrow) and hemorrhage in parenchymal hepatic tissue (blue arrow) with congestion blood vessels (yellow arrow), with inflammatory cells infiltrations and Fatty degeneration of hepatocyte (green arrow). a:10x, b:40x, H&E.

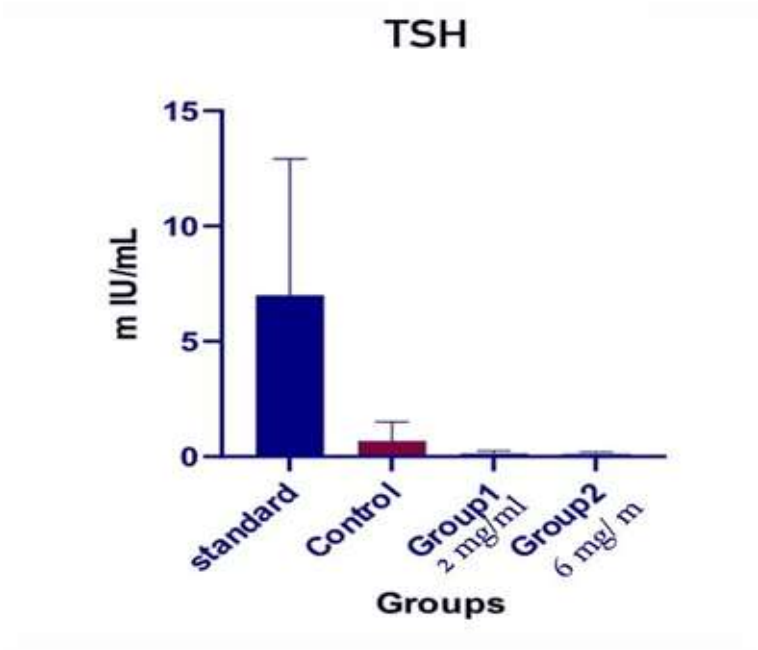

Figure 7. The Effect Of Caffeine on Serum TSH Levels in Adult Male Rabbits

Figure 7 represented the TSH levels in rabbits exposed to caffeine. The current investigation indicates a significant ($p \leq 0.0001$) decrease in serum TSH hormone in Group 1 (0.208333±0.011) treated with 2 mg/ml caffeine and Group 2 (0.014±0.014) treated with 6 mg/ml caffeine compared with the Control Group (0.288333±0.002). TSH is one of the important hormones that have a strong effect on reproductive hormones, so any decrease in TSH levels in serum may have an adverse effect on fertility and cause abnormalities in reproduction. These findings are in line with other previous studies [19], [20]. Furthermore, there were significant decrease in TSH levels in animals that were treated with caffeine, especially those who were exposed high doses of caffeine [21],[22].

The results of figure 8 represented the effects of caffeine on FSH hormone levels. This shows a significant ($p \leq 0.0001$) decrease in serum FSH hormone in Group 1 (0.139167±0.010) that treated with 2mg/ml caffeine, and Group 2 (0.136667±0.120) that received 6 mg/ml compared with the Control Group (0.683333±0.124).

While rabbits exposed to caffeine resulted in a significant ($p \leq 0.0001$) decrease in serum LH hormone in Group 1 (0.184±0.014) that treated with 2 mg/ml, and Group 2 (0.2025±0.042) that dosed with 6 mg/ml compared with the Control Group (0.764167±0.029) as shown in figure 9.

Generally, FSH and LH are the reproductive hormones that play an important role in male reproduction, as FSH regulates the proliferation and maturation of germ cells, and in combination with LH, it triggers and maintains spermatogenesis [23]. It was observed that chronic caffeine administration to animals had an effect on serum FSH and LH, by causing a significant decrease in both serum FSH and TSH levels, which also indicates an inhibition of the spermatogenesis due to caffeine intake; it was also suggested that caffeine intake may influence and effect the secretion of FSH and LH, as documented by early writers [24], [25][26].

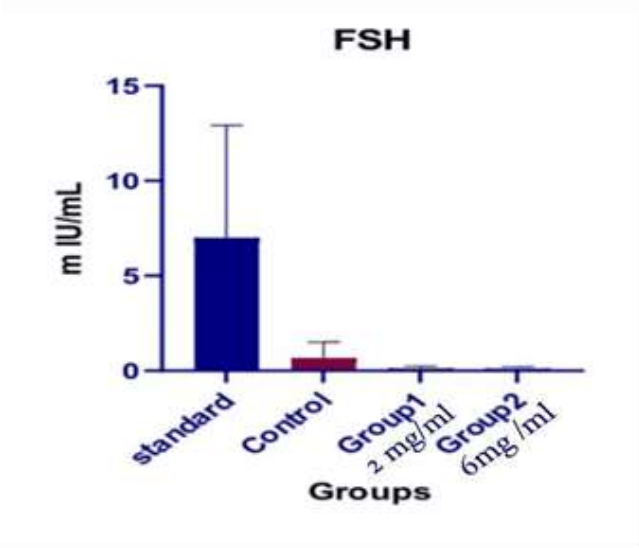

Figure 8. The Effect of Caffeine on Serum FSH Levels in Adult Male Rabbits

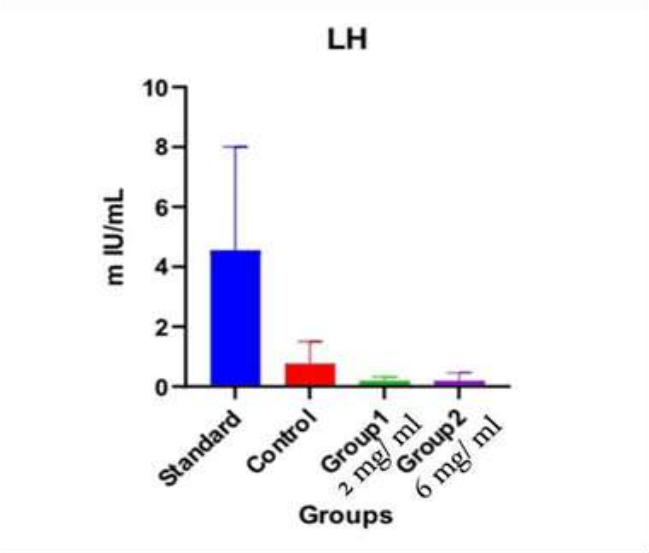

Figure 9. The Effect of Caffeine on Serum LH Levels in Adult Male Rabbits

4. Conclusion

This study has shown that caffeine administration to male rabbits for four consecutive weeks in different concentrations 2 mg/ml and 6 mg/ml both had effects on the testes and liver, and also resulted in a decrease in the reproductive hormones. Administration of caffeine in a concentration of 6 mg/ml, was found to have caused the most severe reaction in both testes and liver.

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Conflict of interest

The authors declare that they have no competing interests.

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