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Effects of excessive administration of caffeine on the size and histology of the testes of adult male Wistar rats

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Abstract

Caffeine is a stimulant commonly added in some tea, coffee, soft drinks and energy drinks. Although, caffeine is widely consumed by both males and females, energy drinks are most commonly consumed among the males (Ricci et. al., 2017; Dillon et al., 20). Moderate consumption may help to reduce the risk of several chronic diseases, including diabetes, liver disease and cancer as well as improve immune functions; however, high consumption (400mg/day) is associated with the risk for the development of cardiovascular diseases (Boyland, 2024). Studies have also shown that high consumption also poses hazards on the reproductive functions of both males and females (Jensen et al., 2020; Ricci et al., 2017). The study aimed to determine the effects of excessive caffeine on the reproductive functions of male wistar rats. Twenty-one-(21) male rats weighing 142-175 grams were used in the study and were divided into 3 groups of 7 animals each. Group A received feed and water only, group B received 25 mg/kg of Caffeine, and group C received 50 mg/kg of Caffeine. The administration of the Caffeine lasted for two-weeks and was administered through oral cannula daily between 6-7am. Data was obtained using the Statistical Science for Social Sciences (SPSS) version 25. Data obtained for relative testicular weight and absolute testicular weight was analyzed using ANOVA followed by post hoc LSD comparison. Body weight was analyzed using T-dependent test. Data was considered significant at $p<0.05$. Result showed that caffeine showed a significant decrease in group B, while group C had no significance. Also, caffeine caused a significant decrease in the absolute and relative testicular weight in groups B and C. Histologically, report showed spermatogenic arrest, decrease active spermatogonia, and necrosis in groups B and C. The study thus revealed that excessive administration of caffeine resulted in negative impact on body weight, testicular weight, and histoarchitectural changes of the testes.

Keywords: Caffeine; Spermatogonia; Testicles; Histology; Spermatogenesis; Reproductive

1. Introduction

The testicle is an organ of the male reproductive tract responsible for spermatogenesis. In mammals, the primary function is to produce sperm and androgens, primarily testosterone, the temperature of the testis must range from 2 to 80 C below body temperature to ensure a successful spermatogenesis. The lower temperature is maintained by a cooling system comprising of the scrotum, pampiniform plexus, and muscles (Senger, 2003). Higher temperatures would lead to an increase of testicular metabolism without a corresponding increase in blood supply, resulting in local hypoxia and deleterious effects for the tissue (Aitken 2008, Ryes 2012). Semen quality and hormonal functions are key determinant of male fertility (Zhao *et al,* 2020), while testosterone and its regulatory hormones have a key factor in spermatogenesis and sperm maturation. Functionally, the leydig and sertoli cells are the important histological cells of the testis with distinct roles in spermatogenesis. Histologically, both cells are pink in the cytoplasm and are identified by pink cyrstals

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of Reinke. Testosterone exerts their effects by binding intracellular receptors of different tissues and regulating protein expression (Gurung & Jialal, 2019). Spermatogenesis and steriodogenesis take place in two compartments that are morphologically and functionally distinguishable from each other, these are the tubular compartment consisting of the seminiferous tubules (tubuli seminiferi) and the interstitial compartment (interstitium) between the seminiferous tubules (Weinbauer, *et al* 2010). Reproductive dysfunction is often noted by malfunction of the reproductive tissues, which could lead to disruption of the synergic rhythm that bring about sexual progression events and the conception. It could result in the sexual dysfunction and infertility seen in couples having prolonged biological struggle in reproducing their offspring after having unrestricted sexual intercourse for at least twelve months (Kayode *et al,* 2020; Kayode & Yakubu, 2017). Several factors have been implicated in the cause and progression of reproductive dysfunction, including poor nutrition, drug side effects, disease states and toxicant ingestion (Beigi Harchegani et al, 2019).

Caffeine is a naturally occurring alkaloid and a stimulant which belong to a class of compounds called methylxanthines, (1,3-7 methylxanthine- C8-H10N4O2)(Frishman *et al*, 2002). It is the most ingested pharmacologically active substance in the world found in common beverages (coffee, tea and soft drinks), medications including headache or pain remedies. Caffeine acts by blocking the binding of adenosine to adenosine A1 receptor, which enhances the release of neurotransmitter acetyl choline (Greenberg et al, 2007). It acts as an antioxidant and helps reduce tissue damage caused by reactive oxygen species (ROS) (Escott-Stump, 2008). Moderate consumption of caffeine may help to reduce the risk of several chronic diseases, including diabetes, liver disease and cancer as well as improve immune function; however, excessive consumption has risk for the development of cardiovascular diseases (Boyland, 2024). It could stimulate the sympathetic nervous system leading to elevated heart rate and blood pressure (hypertension) which is associated with an increased risk of coronary artery disease, heart failure, chronic kidney disease, and dementia (Boyland, 2024). Caffeine is also said to have an effect on the reproductive function (Dorostghoal, 2012), studies have shown that its effects could be exerted on fertility, fecundity, and reproduction (Modi, 2012). This study is designed to compare the weight changes and histology of the testes of male wistar rats after administration of excessive caffeine.

2. Materials and methods

2.1. Experimental Animals

Twenty-one-(21) male rats weighing 142-175 grams were used in the study and obtained from the Animal House of the Department of Physiology, Nnamdi Azikiwe University. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were maintained with normal laboratory chow (Grower feed) and water ad libitium. The animals were acclimatized for two weeks before administering the caffeine. The animals were kept on 12-hours light and dark cycles.

2.2. Ethical approval

Ethical approval was obtained from the ethical committee, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi campus. Rats handling and treatments conform to the rodent handling and restraint (JoVE science education) manual.

2.3. Experimental Protocol

The animals were divided into 3 groups of 7 animals each;

- Group A received feed and water only
- Group B received 25 mg/kg of Caffeine
- Group C received 50 mg/kg of Caffeine

The administration of the Caffeine lasted for two-weeks and was administered through oral cannula daily between 6-7 AM.

1-gram of Caffeine was dissolved in 20mls of warm distilled water to obtain a stock-solution of 50mg/mls. Group B received 25 mg/kg of caffeine, which is equivalent to 0.1ml and group C received 50 mg/kg, which is equivalent to 0.2ml.

Dosage in mls- 0.1ml (Group B); 0.2mls (Group C)

2.4. Termination of experiment and collection of testes

At the end of the experiment following the administration of Caffeine, the animals were anesthetized using chloroform in an enclosed container for 2-minutes after the last administered dose caffeine. The testes were excised from the animals following cervical dislocation and the testes was removed through intrabdominal incision and fixed in Bouin's fluid for histopathological studies.

2.5. Body weight and testicular weight measurement

The body weight and testicular weight was measured using an electronic weighing scale (M-Metllar: M311L). Relative testicular weight was calculated:

2.6. Histopathological Procedure

Tissues (testes) were fixed in bouin's fluid and was dehydrated in four (4) concentrations of Isopropyl alcohol, i.e. 70%, 80%, 90%, 100% for 1hr each and then cleared in xylene before embedded in molten paraffin wax to remove the isopropyl alcohol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues was stained using Haematoxylin and Eosin (H&E) to demonstrate general tissue structure. Tissues sectioned were examined and interpreted using Leica DM 750 binocular microscope with photomicrographic facilities and then photomicrographed by a histopathologist (Ahmed, 2016).

2.7. Statistical Analysis

Data obtained from this study was analyzed using Statistical Science for Social Sciences (SPSS) version 25. Data obtained for relative testicular weight and absolute testicular weight was analyzed using ANOVA followed by post hoc LSD comparison. Body weight was analyzed using T-dependent test. Data was considered significant at $p<0.05$.

3. Results

Table 1 Effect of caffeine on body weight

Groups (N=7)	**Initial body weight (g)**	**Final body weight (g)**	**p-value**	**t-value**
	Mean±SEM	**Mean±SEM**		
Group A (control)	142.00±10.90	163.00±7.64	0.048*	-2.809
Group B (25 mg/kg of caffeine)	167.00±5.65	142.83±2.67	0.002*	5.692
Group C (50 mg/kg of caffeine)	162.00±2.22	151.50±10.42	0.411#	0.897

Data was analaysed using paired t-test and values were considered significant at *p<0.05*. *:significant, #: not significant.

In Table 1, result showed a significant increase in the body weight in group A, while groups B and C had a decrease in the mean weight but showed significance in group B while group C had no significance when the initial weight was compared to the final weight.

Table 2 Effect of caffeine on testicular weight

Groups (N=7)	**Absolute testicular weight (g)**	**Relative testicular weight (g)**
	Mean±SEM	**Mean±SEM**
Group A (control)	2.57±0.13	0.84±0.02
Group B (25 mg/kg of caffeine)	1.87±0.13*	0.64±0.04*
Group C (50 mg/kg of caffeine)	1.59±0.11*	0.51±0.07*
F-ratio	15.917	10.005

Data was analaysed using Analysis of variance (ANOVA) followed by post Hoc LSD comparison and values were considered significant at *p<0.05*. *:significant, #: not significant.

In Table 2, result showed a significant decrease in the mean absolute testicular weight in groups B and C (p=0.004, p=0.000) when compared to group A. The relative mean testicular weight showed a significant decrease in groups B and C (p=0.023, p=0.002) compared to group A.

3.1. Histological Findings

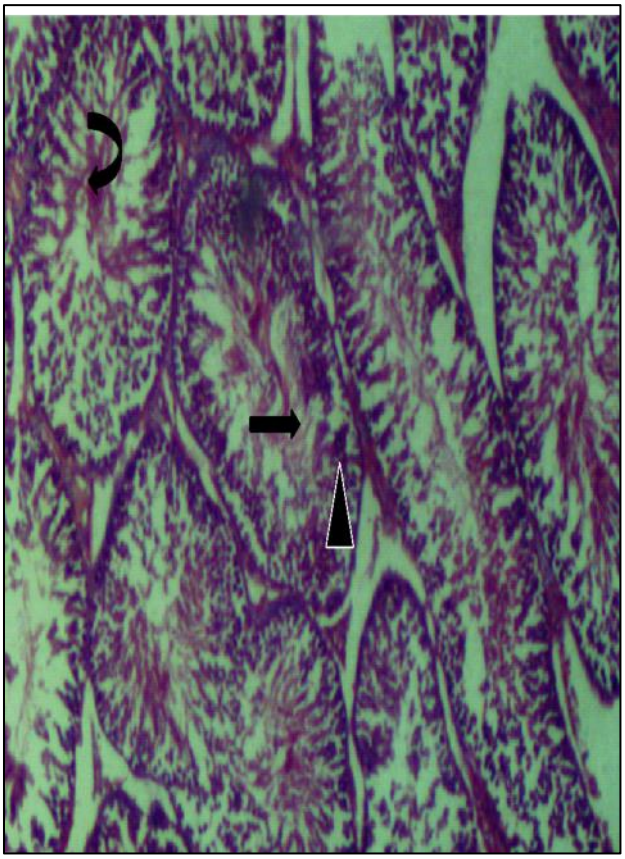

Figure 1 Photomicrograph of a Section of the Testes

Photomicrograph of testicular sections in figure 1 show the ductus epididymis (curved arrow) and the connective tissue appears normal with an increased spermatogenesis. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids with an increased spermatogenesis (arrow). (Stained with H&Ex100).

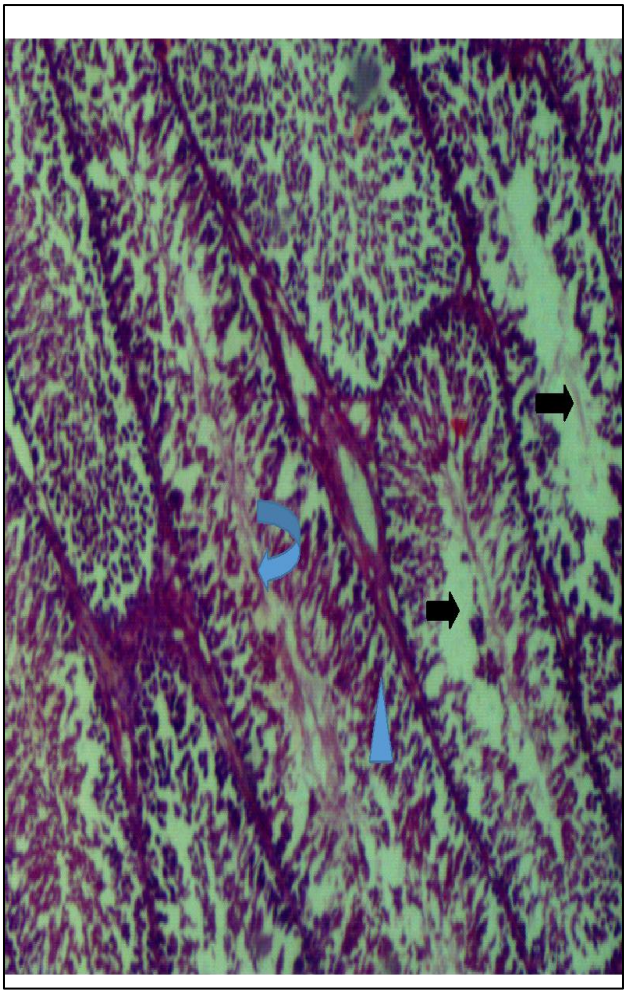

Figure 2 Photomicrograph of a Section of the Testes

Photomicrograph of testicular sections in figure 2 show the ductus epididimys (curved arrow) and the connective tissue appears normal. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids, and mild spermatogenic arrest (arrows). (Stained with H&Ex100).

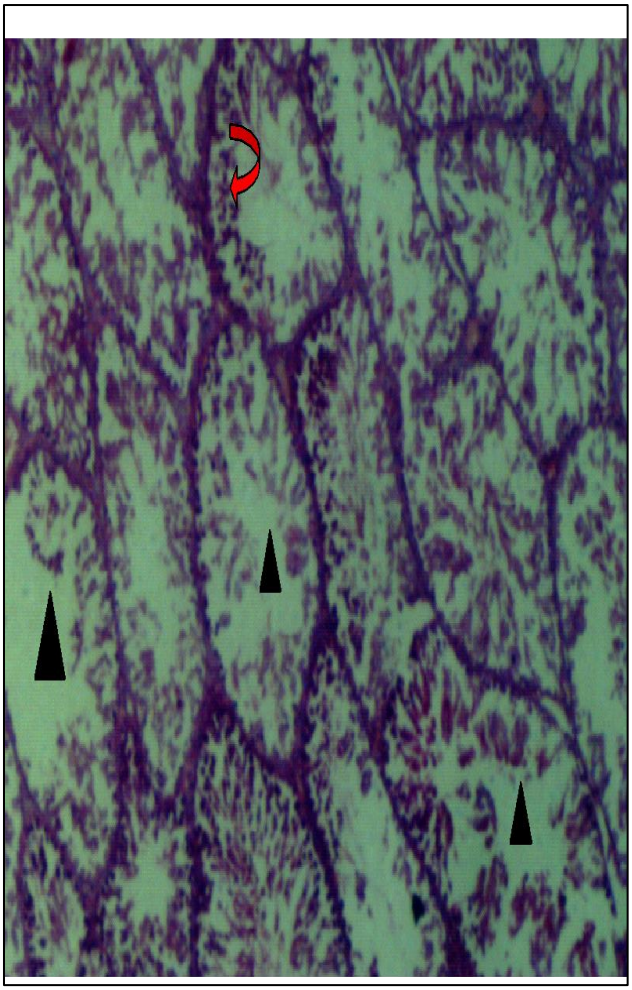

Figure 3 Photomicrograph of a Section of the Testes

Photomicrograph of testiscular sections in figure 3 show scanty spermatids and spermatogonia in the ductus epididymis (curved arrow), severe spermatogenic arrest in the seminiferous tubules (arrowhead). (Stained with H&Ex100).

4. Discussions

Caffeine is one of the most commonly consumed substances, which is present in many plants and products. It has both positive and negative effects on the human body (Rodak *et al.*, 2021). The study investigates the effect of excessive consumption of caffeine on the testes of male Wistar rats. The study findings showed a significant decrease in the body weight in group B, which is linked to oxidative process. However, the physiology linked to the significant decline is generation of reactive oxygen (ROS) species, which alters metabolic processes and energy homeostasis in the hypothalamus. Also, caffeine resulted in the alteration of neurotransmitter that modulates feeding pattern through inhibition of the nerve fibers that regulate satiety centers in the hypothalamus (Rodak *et al*., 2021). The study showed similarity to the findings of Anwar *et al*. (2022) revealing no significant change in the body weight following caffeine intake against diclofenac toxicity in wistar rats. Feyisa *et a*l.(2019), Nassar *et al.*(2013), showed a significant decrease in the body weight gain following caffeine consumption in experimental rats, which agree to the study findings. Tabrizi *et al*.(2019) reported a significant reduction in the body weight gain following caffeine consumption, which is in accordance with the study findings. Haraguchi *et al*.(2022), Park *et al*.(2015), Oluwole *et al*.(2016) and Khan *et al*.(2017) revealed a significant decrease in weight gain in high-fat diet mouse, which agrees to the study findings.

Organ toxicity has been linked to the route of administration to different environmental toxins or endocrine disruptors, which is linked to male dysfunction leading to infertility characterized by other parameters of fertility (Jaishankar, TseteBerginn, Anbalangan, Mathew, and Beeregowda, 2014; Rato and Sousa, 2021). The study showed that administration of caffeine revealed a significant reduction in absolute and relative testicular weight in groups B and C when compared to group A. However, the physiology linked to the significant reduction is associated with the

generation of reactive oxygen species following caffeine metabolism, which causes an increase in the lipid peroxidative process. However, the works of Park *et al.*(2015), Oluwole *et al*.(2016), Kwak *et al*.(2017), Adeyemi *et a*l.(2023), Belali Kharaji *et al*.(2020) revealed a significant decrease in the testicular weight following caffeine consumption, which showed similarity.

The study revealed that histoarchitectural features of the testes following caffeine consumption as indicated in groups B and C demonstrated spermatogenic arrest, decrease active spermatogonia, and necrosis. However, the physiology linked to the negative changes indicated in the seminiferous and Leydig cells of the testes is linked to ROS formation, which causes an increased in the levels of lipid peroxidation within the testicular lipid membrane. However, these changes in the formation of excess unsaturated poly fatty acid chains, results in the formation of free radical species, which causes marked severe degeneration acting through a complex pathway. The study findings showed similar reports to works of Adeyemi *et al.*(2023), Ezim and Abarikwu, (2022), which documented a negative effect of caffeine on the testes following caffeine consumption revealing marked degeneration of seminiferous tubules, decreased spermatogenesis, and spermatogenic arrest. Belali Kharaji *et al.*(2020) and Nkwocha and Ezim, (2024) reported a significant reduction in the spermatogonia cells following caffeine consumption in experimental rats, which agrees to the study report.

5. Conclusion

The study concludes that **excessive** administration of caffeine resulted in negative impact on body weight, testicular weight, and histoarchitectural changes of the testes. However, the effect of caffeine on the body weight, testicular weight, and testicular architecture was dose dependent.

Recommendation

Therefore, it is recommended that excessive consumption of caffeine could result in impaired testicular function in male Wistar rats and would compromise fertility in males both in experimental rats and human's studies. Further, it could be used as an anorexic agent in obese individuals, but its complications or side effects should be put into consideration.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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