

## Excessive Caffeine Intake Disrupts Testicular Architecture, Spermatogenesis and Hormonal Levels in Experimental Wistar Rats

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**Abstract:** Caffeine is popularly consumed and it is a legal psychoactive substance. The effects on testicular structure remains largely unknown. This research was aimed at investigating the effects of caffeine on the testis structure and function. The study was done using 40 Male Wistar rats. The rats were randomly divided into 5 groups. Group A was the control group, Group B was caffeine low dose, Group C was caffeine medium dose, Group D was caffeine high dose, Group E was caffeine very high dose. Group A was administered feed and water for 30 days, Group B was administered 20mg/kg body weight caffeine for 30 days, Group C was administered 50mg/kg body weight caffeine for 30 days, Group D was administered 80mg/kg body weight caffeine for 30 days, Group E was administered 100mg/kg body weight caffeine for 30 days. After the treatment the morphological analysis, histological slides, immunohistochemical, hormonal assay, enzyme assay, and semen analysis were done. Morphological results revealed no significant difference in terms of the relative testis weight between control and treatment groups. Histology and histochemistry revealed aberrations to seminiferous tubule epithelium integrity, glycogen expression using the PAS tests and Placental Alkaline Phosphatase expression in the testes. There were also aberrations in the activities of vital hormones and enzymes that determine fertility. Semen analysis results also complemented the morphological and biochemical results. Results, collectively, showed that high dose caffeine ingestion had compromising effects on fertility, especially, the testis seminiferous tubules integrity, spermatozoa viability as well as hormones and enzymes activities.

**Keywords:** caffeine, testis, fertility, hormone, seminiferous tubules, enzyme

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### I. Introduction

Caffeine (1, 3, 7-trimethyl xanthine) is a natural alkaloid in tea leaves, cocoa beans, kolanuts and certain other plants (Demerec *et al.* 1953; Burkner, 1979). It has been considered to be the most widely consumed drug in human history and it is used by 80% of the world's population and arguably the most commonly ingested psychoactive substance in the world in common beverages including caffeinated soft drinks and medication, including headaches or pain remedies and over-the-counter stimulants. Caffeine stimulates the central nervous system (CNS) and alters mood and behaviour. Caffeine ingestion is mostly attributed to the consumption of coffee (140mg/cup), tea (47mg/cup), caffeinated soft drinks (up to 80mg can) as well as some drugs and medication (Rall, 1980); hence, complete non-consumers of caffeine may therefore be close to non-existence (Rither *et al.*, 1999). Caffeine, theobromine and theophylline found in kola nut are xanthine stimulants (Somorin, 1973) and the caffeine content of kola nut could be often responsible for the physiological or clinical effect of kola nut in humans and mammals generally (Ogutuga 1975; Chukwu *et al.*, 2006). Caffeine was first isolated from green coffee beans in 1820 and in tea mate and kola nuts. (Burkner, 1979) are the main sources of caffeine. A wide variety of values for caffeine content have been reported, especially in coffee (Barone, 1984). Caffeine content of natural products varies based plant species, environmental conditions, method of preparations (Barone & Roberts, 1984)

The liver is the primary site of caffeine metabolism (Huxlacy, 2009). Coffee is one of the most widely consumed beverages in the world (Afman, 2000). More recently, coffee consumption has been associated with a reduction in the risk of several chronic diseases, including type 2 diabetes mellitus, Parkinson's disease and liver disease (Bode, 2007). One of the benefits of caffeine is also reported to include reduction in cholesterol, bile acid, and neutral sterol secretion in the colon is a direct effect of coffee consumption as is increased colonic motility, which can reduce exposure of epithelium to carcinogens (Shapiro *et al.*, 2008). On the other hand, a number of negative health effects of caffeine have been studied. Short term side effects include headache,

nausea, and anxiety (Marshall,2000). Caffeine competitively inhibits different adenosine receptors and their associated G protein to make a person feel alert. Caffeine also stimulates cardiac muscle, causes relaxation of the smooth muscle, increase in gastric secretions, and diuresis. (Afman *et Al.*, 2000). Caffeine consumption may affect skeletal growth (Bakker *et al.*, 2010) and cause low birth weight (Bracken *et al.*, 2003). Caffeine consumption affected fetal growth and altered reproductive functions (Dorostghoal, 2012). Yet caffeine-containing foods and beverages are consumed by adults and children (Gilbert, 1984).

While many existing literatures have addressed the effects of caffeine on female fertility, conception, fecundity and birth weight as well as foetal health, it is very important to consider the effects of caffeine on male fertility. The primary organ in the male reproductive system is the testis. The testis produces spermatozoa in a process known as spermatogenesis. It is therefore important to understand how caffeine affects the testis structural integrity. The testicle is the male gonad in animals. Like the ovaries to which they are homologous, testes are components of both the reproductive system and the endocrine system. The primary functions of the testes are to produce sperm ( spermatogenesis) and to produce androgens, primarily testosterone (Blackwell, 2010). Both functions of the testicle are influenced by gonadotropic hormones produced by the anterior pituitary. Luteinizing hormone (LH) results in testosterone release. The presence of both testosterone and follicle-stimulating hormone (FSH) is needed to support spermatogenesis. It has also shown in animal studies that testes are exposed to either too high or too low levels of estrogens spermatogenesis can be disrupted to such an extent that the animals become infertile (sierens, 2005). Almost all healthy male vertebrates have two testicles. They are typically of similar size, although in sharks, that on the right side is usually larger, and in many bird and mammal species, the left may be the larger. The primitive jawless fish has only a single testis, located in the midline of the body, although even this forms from the fusion of paired structures in the embryo (Romer, 1977).

Structurally, the testis consists of a number of seminiferous tubules within which spermatogenesis take place and the entire testis is covered by the tunica albuginea. Beneath the tough tunica albuginea, the testis contains the very fine coiled tubes called seminiferous tubules. The tubules are lined with a layer of cells (germ cells) that from puberty into old age, develop into sperm cells or spermatozoa. The matured spermatozoon travels through the seminiferous tubules to the rete testis located in the mediastinum testis, to the efferent ducts, and then to the epididymis where newly created sperm cells mature. The spermatozoon further travels into the vas deferens, and are eventually expelled through the urethra and out of the urethral orifice through muscular contractions during ejaculation (Romer,1977).

Caffeine has been reported to have certain negative effects on fertility, fecundity or reproduction. Caffeine and of its metabolites have been suggested as risk factors in birth defect (Jacobson *et al.*, 1981), heart disease (Heyden *et al.*,1978) and pancreatic cancer. The popularity of caffeine is due to its stimulant action. Epidemiological studies have been advocated as the best approach to exploring any link between caffeine and reproductive outcome (Olsen, 1991). Caffeine has been reported as a risk factor for delayed conception (Wilcox *et al.*, 1988). Caffeine consumption has also been implicated as a risk factor for spontaneous abortions ( Fenster *et al.*, 1991).

## II. Materials And Methods

### Animas Grouping and Treatment

Adult male Wistar rats with an average body weight of 100g – 200g were purchased from animal house of Babcock University, Ilishan-Remo All animals were fed with commercially formulated rat chow and water. The animals were left to acclimatize in Babcock University's animal house for a period of 2 week. Ethical Approval for this study would be obtained from the Babcock University Research Ethical committee of Babcock University, Ilishan –Remo. The 40 Wister rats were weighed at the end of acclimatization period after which they were grouped into 5; with each group having 8 rats each, the five groups are as follows:

- Group A- the control group, this group was fed with pellet and water only *ad libitum*.
- Group B- this group was administered 20 mg/kg body weight caffeine using an oral cannula for a period of four weeks.
- Group C- this group was administered 50mg/kg body weight caffeine using an oral cannula for a period of four weeks.
- Group D- this group was administered 80 mg/kg body weight caffeine using an oral cannula for a period of four weeks.
- Group E- this group was administered 100 mg/kg body weight caffeine using an oral cannula for a period of four weeks

The experimental groups B,C,D,E were fed with pellet, water and caffeine respectively. During the course of the experiment, the rats were weighed on a weekly basis in order to monitor their weights and to know if there are any changes in body weight.

### **Animal Sacrifice**

At the end of the experiment, the animals were sacrificed through cervical dislocation. Blood samples were collected withdrawn through ocular puncture and appropriately dispensed into EDTA bottles for serum testosterone, Follicle stimulating hormone and luteinizing hormone analysis. The testis and epididymis was excised, the testis was weighed and fixed in 10% formalsaline, 10% sucrose solution in separate sample bottles , the epididymis was preserved in normal saline for semen analysis

### **Procedure for Sperm Analysis**

The testicles and epididymis were exposed through a lower abdominal incision. The epididymis were excised from the body of the testes. Sperm cells from the caudal were released into a petri dish containing normal saline to liquefy and migrate all spermatozoa from the epididymal tissue to the fluid (Morankinyo *et al.*, 2010). Semen analysis was done according to Zemjanis's diagnostic and therapeutic techniques in animal reproduction (WHO, 1987)

### **Sperm Morphology**

Smear was prepared for each rat from the collected sperm cells and stained with Eosin solution. Sperm cells with no hook-shaped heads and/or were not elongated were considered abnormal. A minimum of 100 spermatocytes per slide were examined and cell morphology was evaluated under the light microscope.

### **Sperm Motility**

Cell motility was recorded and evaluated immediately after tissue isolation. A drop of sperm cells was dropped on a glass slide, covered with slip and then examined under a light microscope (Olympus). The sperm cells that were not moving at all were at all were considered immotile while those that displayed some movements were considered to be motile. The percentage sperm motility was calculated using the number of live sperm over the total number of sperm cells.

### **Sperm count**

Sperm count was done under the microscope using improved Neubauer haemocytometer. Sperm cells were counted in five diagonal large Thomas squares and the concentration of sperm in cells/mL was calculated using this formular:

Dilution factor × Count in 5 squares × 50,000

### **Sperm Viability**

Sperm viability was immediately assessed and recorded after tissue isolation. A mixture of semen, eosin and nigrosin was made by mixing one drop of semen with two drops of 1% of eosin followed by three drops of 10% nigrosin solution. Within 30n seconds of making mixture, a smear was made on microscope slide, air dried and examined under oil immersion ( X 1000) using a light microscope. The percentage sperm viability was calculated using the number of live (white) sperm over total number of dead (pink/red) sperm cells.

Caudal part of the rat's epididymis was excised and put into 0.9% normal saline , each of the excised epididymis was minced with 1ml of normal saline in order to evaluate sperm count, sperm morphology, and super motility, Sperm count analysis, sperm motility and morphology analysis

### **Blood Collection**

Blood was gotten by way of ocular puncture through heparinised cappillary tubes and put into the blood sample bottles. This was immediately put into a bucket with ice blocks for preservation. The blood was later taken and centrifuged for about 15 minutes. After the serum had been separated from the red cells, it was stored in bottles and sealed before being put in the refrigerator. The serum was later taken for FSH, LH, testosterone assays.

### **Histological Studies Procedure: Haematoxylin and Eosin Staining Protocol**

Tissues processing refers to the series of things that has been done to a fixed tissue before it can be embedded with paraffin wax for sectioning. This is the most common staining technique in histology. It uses a combination of the haematoxylin and eosin dyes to demonstrate the nucleus and cytoplasmic regions.

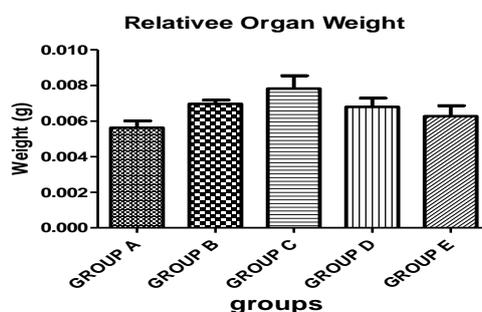
The tissue section was deparaffinised, followed by hydration [the tissue is hydrated by passing through decreasing concentration of alcohol baths and water ( 100%, 90%, 80%, 70%)]. The tissues were stained in hematoxylin for 3 to 5 minutes; then, washed in running tap water until the sections "blue" for 5 minutes or less. They were differentiated in 1% acid alcohol ( 1% HCL in 70% alcohol) for 5 minutes and washed in running tap water until the sections are blue again by dipping in an alkaline solution followed by tap water wash. Sections were stained in 1% eosin for 10 minutes and washed in tap water for 1 to 5 minutes. Sections were dehydrated in increasing concentration of alcohols and clear in xylene.

Lastly, samples were mounted in mounting media (dpx) and observed under microscope.

### III. Results

#### Relative Organ Weight (ROW)

The ROW for Group A (control group) was  $0.0056 \pm 0.0004$ . The ROW of Group B (caffeine low dose group) was slightly higher at  $0.0070 \pm 0.0002$ . The ROW of Group C (caffeine medium dose group) was slightly higher than the control at  $0.0078 \pm 0.0007$ . The ROW of Group D (caffeine high dose group) was slightly higher than control at  $0.0068 \pm 0.0005$ . The ROW of Group E (caffeine very high dose group) was slightly higher than control at  $0.0063 \pm 0.0006$

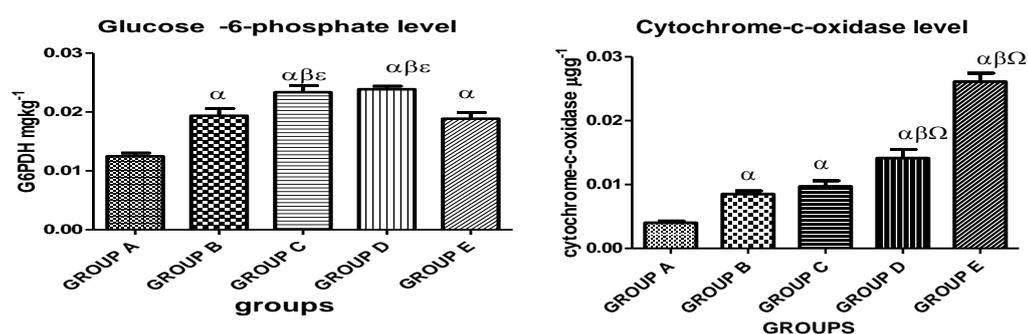


#### Cytochrome-C-Oxidase

The cytochrome-c-oxidase level for Group A (control group) was  $0.004 \pm 0.0003$ . The cytochrome-c-oxidase level for Group B (caffeine low dose group) was significantly ( $P < 0.05$ ) higher at  $0.009 \pm 0.0005$  when compared with the control group. The cytochrome-c-oxidase level for Group C (caffeine medium dose group) was significantly ( $P < 0.05$ ) higher at  $0.010 \pm 0.0009$  when compared with control group. The cytochrome-c-oxidase level for Group D (caffeine high dose group) was significantly ( $P < 0.05$ ) higher at  $0.014 \pm 0.001$  when compared with control group. The cytochrome-c-oxidase level for Group E (caffeine very high dose group) was significantly ( $P < 0.05$ ) higher at  $0.026 \pm 0.001$  when compared with control group.

#### Glucose-6-Phosphate Dehydrogenase (G-6-PDH)

The G6PDH level for Group A (control group) was  $0.013 \pm 0.0005$ . The G-6-PDH level for Group B (caffeine low dose group) was significantly ( $P < 0.05$ ) higher at  $0.019 \pm 0.001$  when compared to the control group. The G6PDH level for Group C (caffeine medium dose group) was significantly ( $P < 0.05$ ) higher at  $0.02 \pm 0.001$  when compared to the control group. The G6PDH level for Group D (caffeine high dose group) was significantly ( $P < 0.05$ ) higher at  $0.02 \pm 0.001$  when compared to the control group. The G-6-PDH level for Group E (caffeine very high dose group) was significantly ( $P < 0.05$ ) higher at  $0.02 \pm 0.001$  when compared to the control group.



**Figure 4.5** Bar chart showing the cytochrome-c-oxidase levels of control and treated groups after 30 days of treatment [ $\alpha = P < 0.05$  when compared with Group A;  $\beta = P < 0.05$  when compared with Group B;  $\Omega = P < 0.05$  when compared with Group C]

#### Hormone Assay: Testosterone

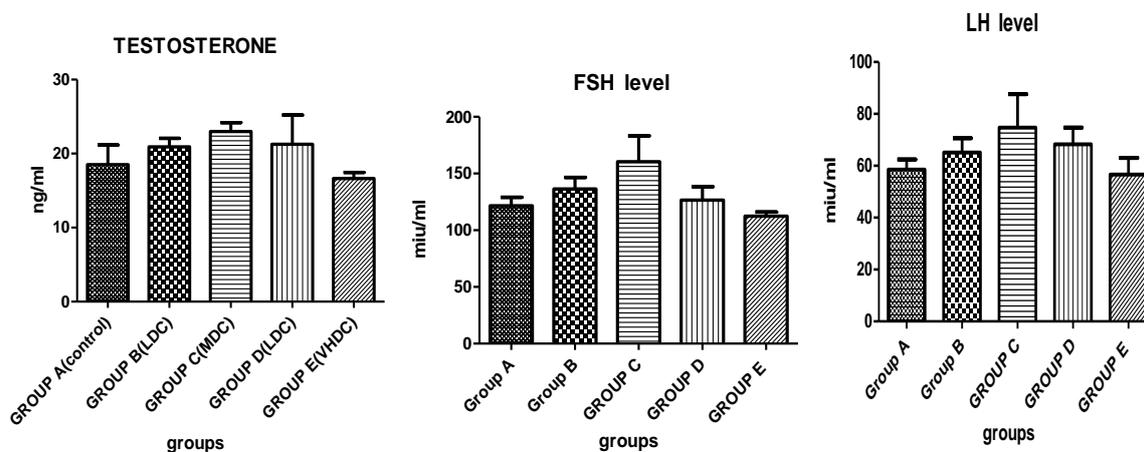
The testosterone level for Group A (control group) was  $18.52 \pm 2.631$ . The testosterone level for Group B (caffeine low dose group) was slightly higher than control group at  $20.90 \pm 1.153$ . The testosterone level for Group C (caffeine medium dose group) was slightly higher when compared to control group at  $22.99 \pm 1.198$ . The testosterone level for Group D (caffeine high dose group) was slightly higher when compared to control group at  $21.25 \pm 3.960$ . The testosterone level for Group E (caffeine very high dose group) was lower when compared with control group at  $16.64 \pm 0.838$ .

**Follicle Stimulating Hormone (FSH)**

The FSH level for Group A (control group) was  $121.6 \pm 7.58$ . The FSH level for Group B (caffeine low dose group) was slightly higher than control at  $136.4 \pm 10.11$ . The FSH level for Group C (caffeine medium dose group) was higher when compared with control group at  $160.3 \pm 22.94$ . The FSH level for Group D (caffeine high dose group) was slightly higher when compared with control group at  $126.5 \pm 11.96$ . The FSH level for Group E (caffeine very high dose group) was lower than control group at  $112.4 \pm 3.65$

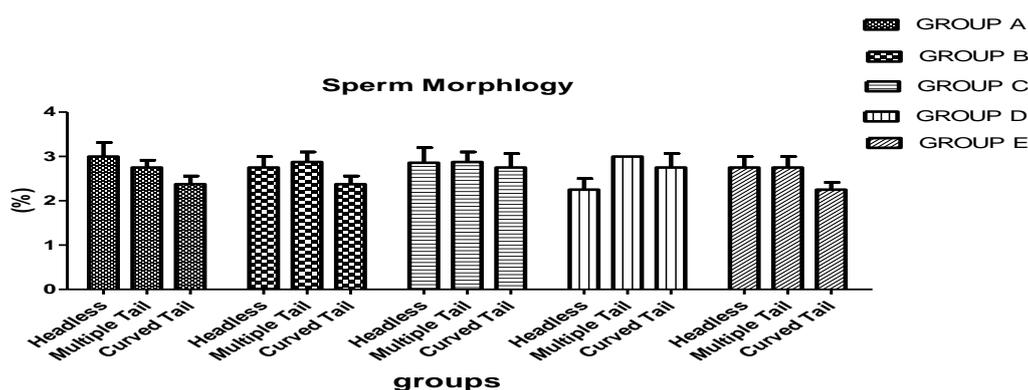
**Luteinising Hormone (LH)**

The LH level for Group A (control group) was  $58.55 \pm 3.92$ . The LH level for Group B (caffeine low dose group) was slightly higher than control group at  $65.11 \pm 5.49$ . The LH level for Group C (caffeine medium dose group) was higher when compared with control group at  $74.74 \pm 12.89$ . The LH level for Group D (caffeine high dose group) was slightly higher when compared with control group at  $68.29 \pm 6.46$ . The LH level for Group E (caffeine very high dose group) was slightly lower when compared with control group at  $56.60 \pm 6.42$



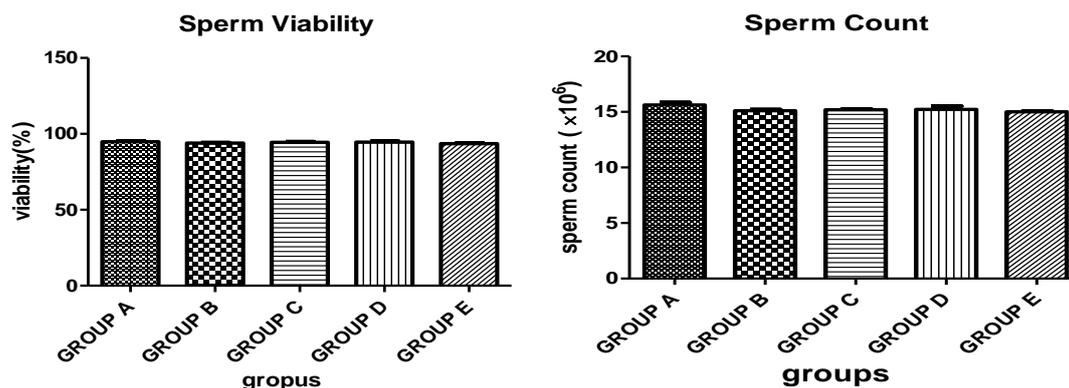
**Sperm Analysis: Sperm Morphology (SM)**

The SM level for Group A (control group) was  $90.05 \pm 31.51$ . The SM level for Group B (caffeine low dose group) was slightly higher than control group at  $100.8 \pm 35.65$ . The SM level for Group C (caffeine medium dose group) was higher when compared with control group at  $117.5 \pm 42.80$ . The SM level for Group D (caffeine high dose group) was slightly higher when compared with control group at  $97.40 \pm 29.12$ . The SM level for Group E (caffeine very high dose group) was slightly lower when compared with control group at  $84.48 \pm 27.88$ .



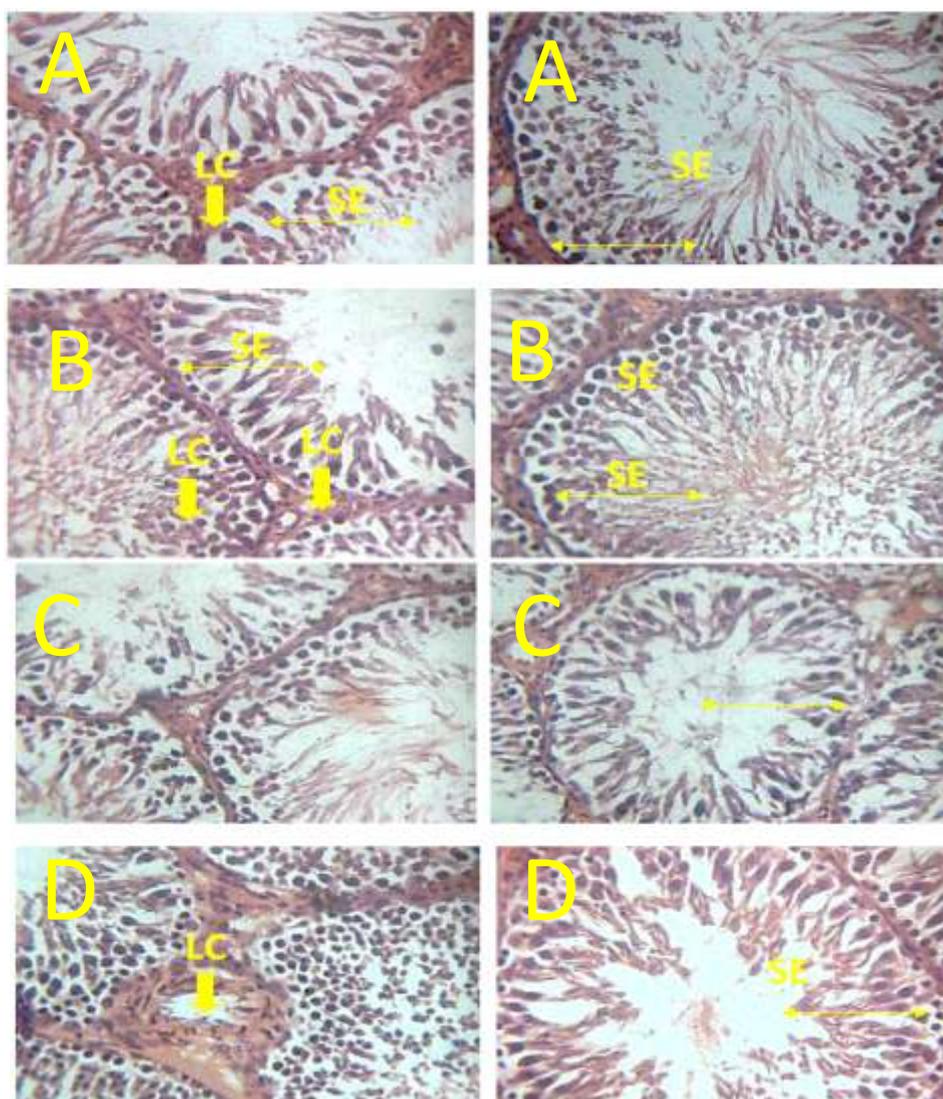
**Sperm viability (SV)**

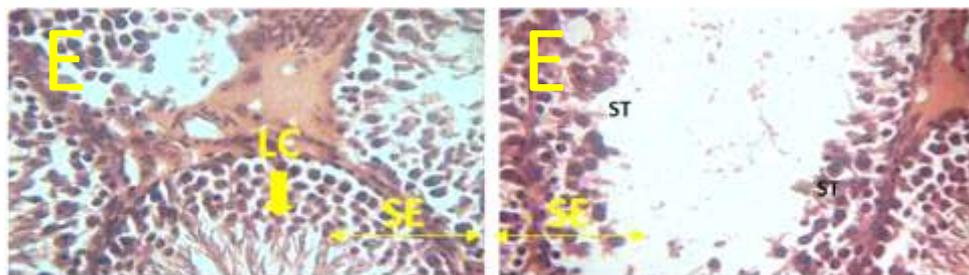
The SV level for Group A (control group) was  $94.80 \pm 0.6$ . The SV level for Group B (caffeine low dose group) was slightly lower than control group at  $93.88 \pm 0.48$ . The SV level for Group C (caffeine medium dose group) was slightly lower when compared with control group at  $94.43 \pm 0.48$ . The SV level for Group D (caffeine high dose group) was slightly lower when compared with control group at  $94.50 \pm 0.65$ . The SV level for Group E (caffeine very high dose group) was slightly lower when compared with control group at  $93.50 \pm 0.57$



**Sperm Count (SC)**

The SC level for Group A (control group) was  $15.62 \pm 0.24$ . The SC level for Group B (caffeine low dose group) was slightly higher than control group at  $15.11 \pm 0.11$ . The SC level for Group C (caffeine medium dose group) was higher when compared with control group at  $15.19 \pm 0.1$ . The SC level for Group D (caffeine high dose group) was slightly higher when compared with control group at  $15.23 \pm 0.3$ . The SC level for Group E (caffeine very high dose group) was slightly lower when compared with control group at  $15.0 \pm 0.1$ .





**Figure1:** Photomicrographs of the testis structure of the experimental animals in Group A-E [H & E; X1600] demonstrating the seminiferous tubules. Seminiferous epithelium integrity is compromised increasingly with increase in caffeine dosage.

### Histological Result [Figure 1]

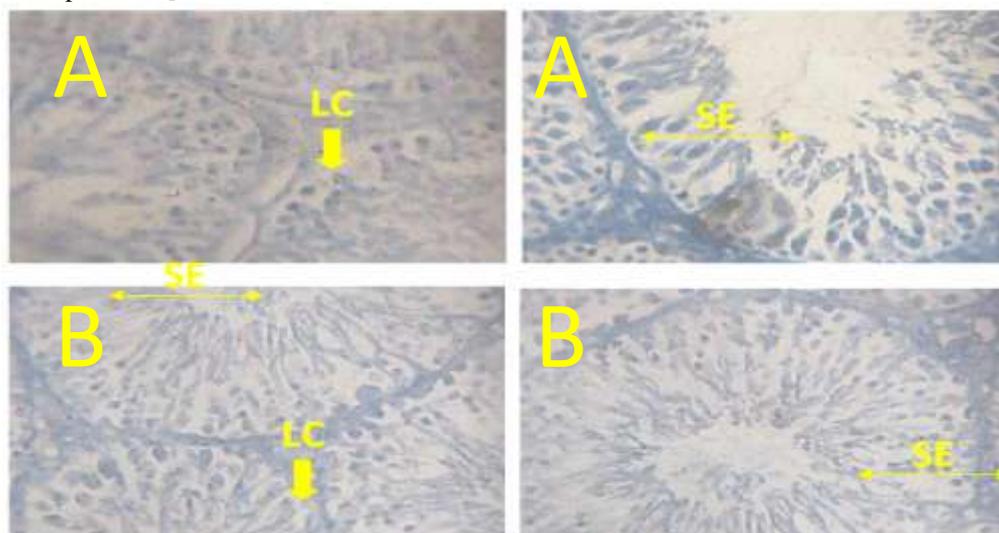
Histoarchitectural features of the testis shows that in Group A seminiferous tubules are normally demonstrated and distributed. Tubules are normal and the primordial spermatogenic cells are normally distributed within the seminiferous epithelium. Leydig cells are demonstrated with normal morphologies and spatial distribution; in Group B, seminiferous tubules morphologies are relatively normal in the treated animals. In Group C, seminiferous tubules are mildly distorted. In Group D seminiferous tubules are distorted and Leydig cells are destroyed. In Group E seminiferous tubules are extensively destroyed and Leydig cells are extensively destroyed. [Legend: ST- Seminiferous Tubule, LC- Leydig Cells, SE- Seminiferous Epithelium]

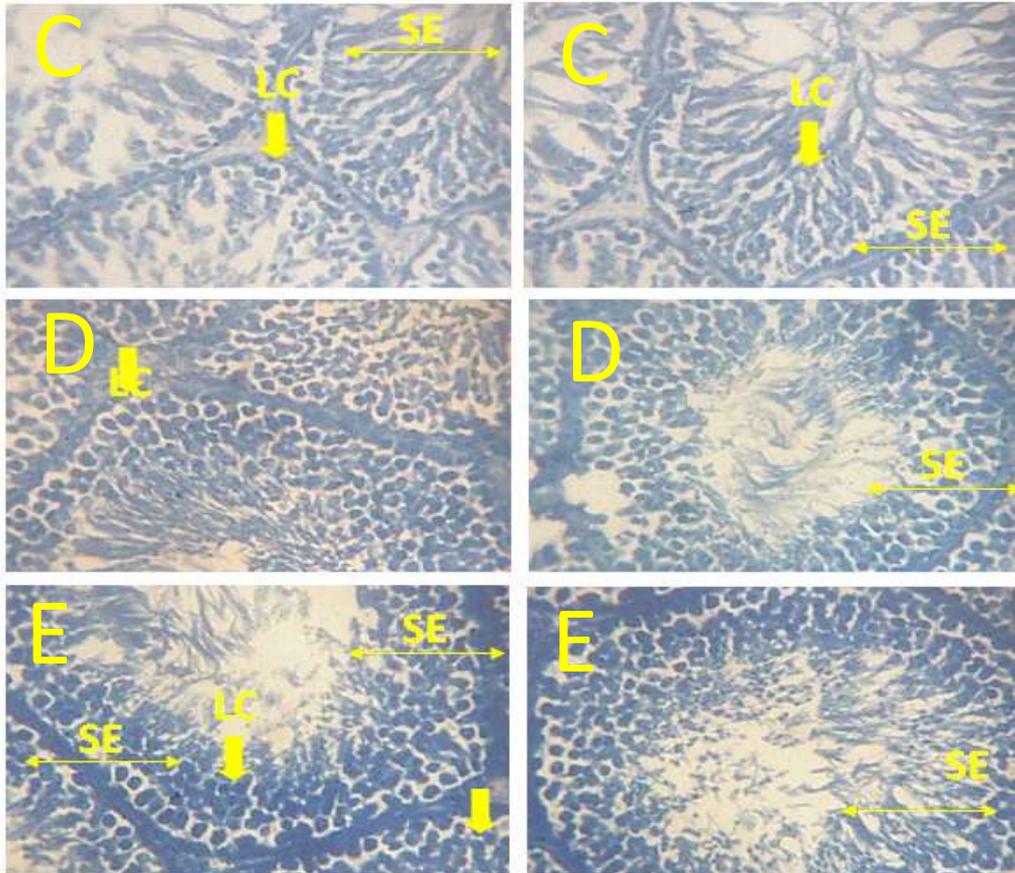
### Periodic Acid-Schiff [PAS] Histochemistry Results [Figure 2]

Photomicrographs of the testis structure of the experimental animals in Group A showed Periodic Acid-Schiff [PAS] normal demonstration of glycogen and mucosubstances in the testicular tissues as a test for glycogen storage and glucose metabolism functional integrity of the tissue. Photomicrographs of the testis structure of the experimental animals in Group B showing Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testicular tissues. PAS expression is relatively normal. In Group C, Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testis. PAS expression is relatively normal. In Group E Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testis seminiferous epithelium. PAS expression is relatively more intense. In Group E PAS demonstration in tissue is relatively intense; and this observation co-exists with seminiferous epithelium disruption. [Legend: LC- Leydig Cells; SE- Seminiferous Epithelium]

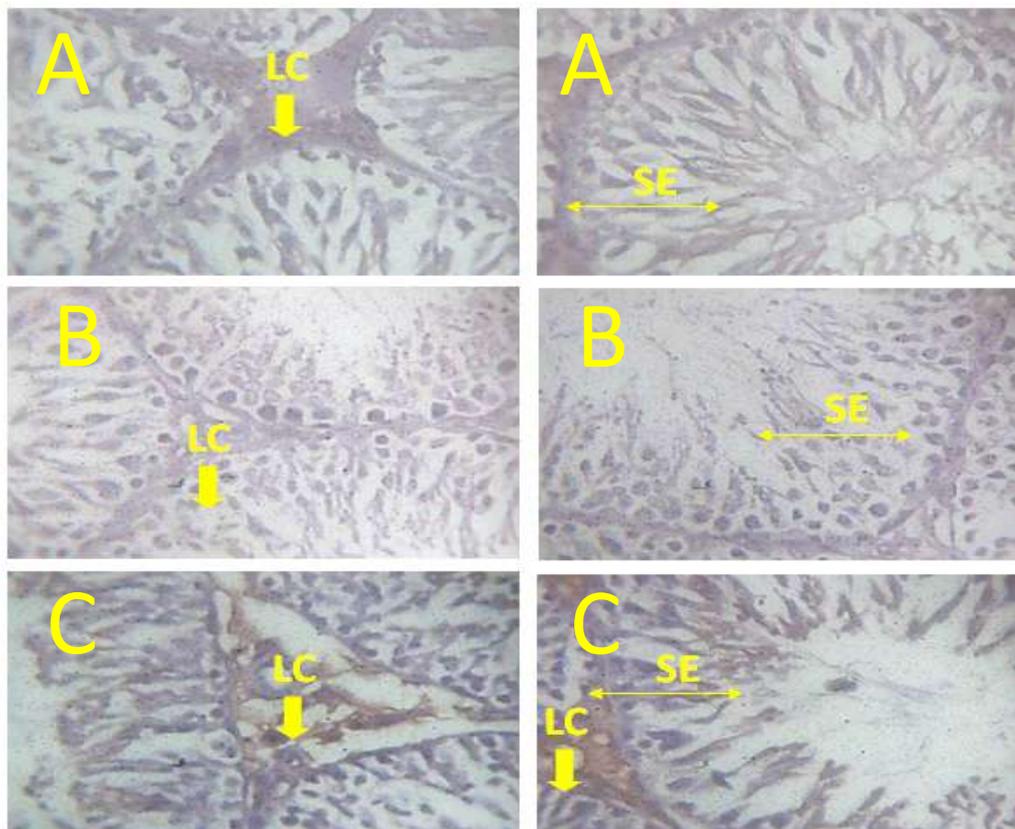
### PLAP [Placental Alkaline Phosphatase] Immunohistochemistry Result [Figure 3]

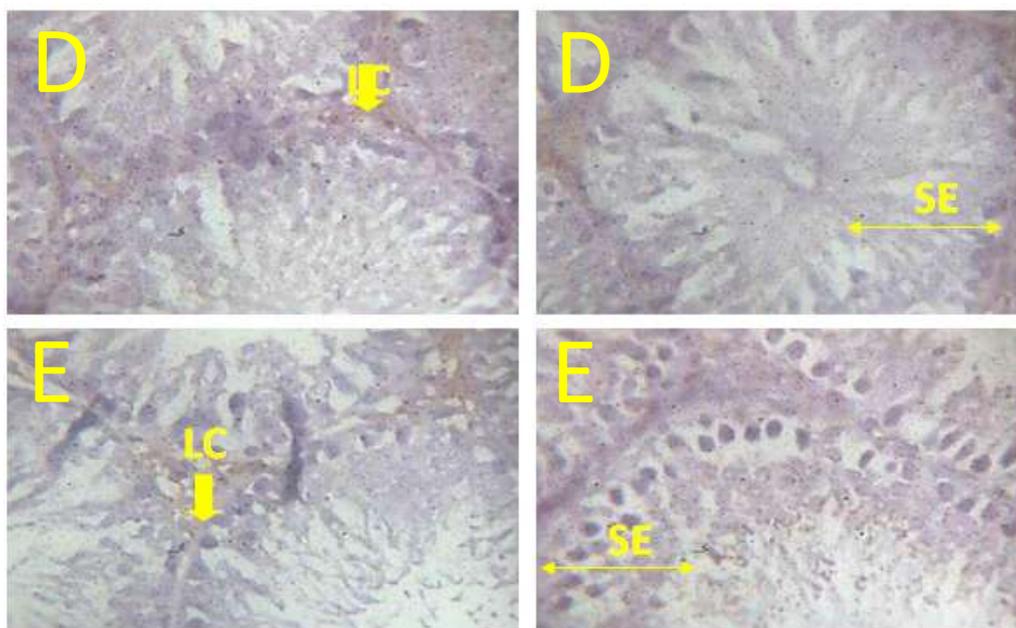
The photomicrographs of the testis PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups A to observe possible testicular germ cell tumours or evidences of molecular assaults [PLAP] showed that germ cells and interstitial Leydig cells did not stain positive for PLAP. In Groups B, germ cells and interstitial Leydig cells did not stain positive for PLAP. In Group C interstitial Leydig cells stain mildly positive for PLAP. In Groups D interstitial Leydig cells stain mildly positive for PLAP. In Groups E interstitial Leydig cells stain mildly positive for PLAP. Apical cells of the reduced and distorted seminiferous epithelium also mildly express PLAP. [Legend: LC- Leydig Cells; SE- Seminiferous Epithelium]





**Figure 2:** Photomicrographs of the testis structure of the experimental animals in Group A-E [PAS; X1600] demonstrating the seminiferous tubules. PAS expression in the seminiferous epithelium increased with increase in caffeine dosage.





**Figure 3:** Photomicrographs of the testis structure of the experimental animals in Group A-E [PLAP; X1600] demonstrating the seminiferous tubules. PLAP expression is only observable in the groups administered the high caffeine doses, though mildly.

#### IV. Discussion

Testicular structure was studied using the haematoxylin and eosin staining technique. The seminiferous tubules integrity in terms of their sizes and structural organisation with respect to the wall and the inner lining of seminiferous epithelium are considered to be for determining the structural integrity of the testes in the experimental animals tissues. The interstitial spaces have Leydig cells in connective tissue stroma; the former provides information on hormonal functions of the testis.

##### General Structural Integrity

Photomicrographs of the testis structure of the experimental animals in Group A show testicular seminiferous tubules that are normally demonstrated and distributed. Tubules are normal and the primordial spermatogenic cells are normally distributed within the seminiferous epithelium. Leydig cells are demonstrated with normal morphologies and spatial distribution. The testis in this group is thus healthy, normal and good for a standard reference.

Photomicrographs of the testis structure of the experimental animals in Group B show seminiferous tubules morphologies that are normal, relative to control. The testicular tissue of the Group C animals - represented by photomicrographs show that seminiferous tubules are mildly distorted. Photomicrographs of the testis structure of the experimental animals in Group show that the seminiferous tubules are distorted and Leydig cells are destroyed. In Figure 4.5, the photomicrographs of the testis structure of the experimental animals in Group E show that the seminiferous tubules are extensively destroyed and Leydig cells are extensively destroyed.

These findings showed that increased or high caffeine dose was deleterious to testicular seminiferous tubules and the Leydig cells in manner that was dose dependent.

##### Functional Integrity

The Periodic Acid-Schiff [PAS] was used for the demonstration of glycogen and mucosubstances in the testicular tissues as a test for glycogen storage and glucose metabolism functional integrity of the tissue. The photomicrographs of the testis structure of the experimental animals in Group E shows Periodic Acid-Schiff [PAS] normal demonstration of glycogen and mucosubstances in the testicular tissues as a test for glycogen storage and glucose metabolism functional integrity of the tissue. The tissue is normal. Photomicrographs of the testis structure of the experimental animals in Group E also show that the Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testicular tissues. PAS expression is relatively normal.

Photomicrographs of the testis structure of the experimental animals in Group E show that Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testis portrays a relatively normal PAS expression relative to the control.

Photomicrographs of the testis structure of the experimental animals in Group E show the Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testis seminiferous epithelium; and PAS expression is relatively more intense. Photomicrographs of the testis structure of the experimental animals in Group E indicate Periodic Acid-Schiff [PAS] demonstration of glycogen and mucosubstances in the testicular tissues of the group. PAS demonstration in tissue is relatively intense; and this observation co-exists with seminiferous epithelium disruption.

The above observations are consistent with the structural changes that were observed in the same groups. Caffeine, therefore at the high doses, had a negative effects on the animals' testes by altering the PAS-expression of glycogen and mucosubstances.

### **Immunohistochemistry of Testicular Tissue**

PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups A was employed to observe possible testicular germ cell tumours or evidences of molecular assaults. Photomicrographs of the testis PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups A showed that germ cells and interstitial Leydig cells did not stain positive for PLAP; the tissue is normal.

Photomicrographs of the testis PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups B showed that germ cells and interstitial Leydig cells did not stain positive for PLAP. The tissue in this group is also normal. Photomicrographs of the testis PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups C showed certain interstitial Leydig cells staining mildly positive for PLAP. This indicated a deleterious effect of caffeine on the testicular Leydig cells in this group.

Photomicrographs of the testis PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups D indicated interstitial Leydig cells that stained mildly positive for PLAP also. Photomicrographs of the testis PLAP [placental alkaline phosphatase]- Immunohistochemistry demonstration of the experimental animals in Groups E has interstitial Leydig cells stain mildly positive for PLAP. Apical cells of the reduced and distorted seminiferous epithelium also mildly express PLAP.

A number of literatures in humans and experimental models have reported either the tendencies or actual negative effects of caffeine ingestion on male fertility, especially semen and sperm qualities and testicular integrity (Jensen *et al.*, 2010; Ricci *et al.*, 2017). Even maternal consumption of caffeine has been linked to compromised fertility in the male offspring of experimental rats (Dorostghoal *et al.*, 2012). These evidences share similarities to various extents with the current study; however, only at the very high doses. Furthermore, it appears increasingly clear that effects are dose-dependent and deleterious effects might not be observed at relatively low doses. Hence, reports should emphasise the roles of dose variations and experiments should model human scenarios of caffeine ingestion to obtain reliable and relevant results.

## **V. Conclusion**

The above results showed that high caffeine dose affected the testicular germinal epithelium structure negatively. These effects would also affect fertility negatively. Thus, caffeine at high dose affected testicular structural integrity negatively. Deleterious effects were not observed at the lower doses of caffeine employed in this experiment.

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