



## Coffee and Caffeine Consumption in Reproductive Functions of Adult Wistar Rats

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### Authors' contributions

This work was carried out in collaboration between all authors. Author PRCE carried out the bench work. Authors AAA, ODE and RAA wrote the first draft of the manuscript and performed the statistical analysis. Authors OMO and AON managed the literature searches. Author JCI designed and supervised the study. All authors read and approved the final manuscript.

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### ABSTRACT

Coffee from *Coffea arabica* is a popular beverage consumed worldwide. Its effect on health has been a global puzzle. In this study, the effect of coffee and caffeine consumption on some reproductive structures and functions of Wistar rats were investigated. A hundred and seventy-five (175) Wistar rats bred in the Animal house of the Faculty of Basic Medical Science of Delta State University were used for this study. All experimental rats were treated for four (4) weeks. Group 1, control, received food and water only, groups 2, 3 and 4 received 40 mg/kg, 60 mg/kg and 80 mg/kg, doses of Coffee respectively while Groups 5, 6 and 7 received 30 mg/kg, 45 mg/kg and 60

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mg/kg doses of Caffeine respectively. After administration of test substances, animals were sacrificed accordingly with serum samples collected. While testes, hypothalamus and hippocampus were harvested for histological studies, serum samples were analysed for specific parameters. In Unit two (2) Seventy-five (75) rats of both sexes were mated and treated in varying mating and treatment groups accordingly until after gestation. Then, the weight, litter size, survival rate and gestation length were measured. Both Caffeine and Coffee treatments showed a dose-dependent effect on most parameters measured. Coffee was found to increase antioxidant enzymes, decrease liver enzymes and also negatively affected reproductive outcome. All comparisons were done at ( $P \leq 0.05$ ), using 1-tailed ANOVA.

*Keywords: Coffea arabica; anti-oxidants; beverages.*

## 1. INTRODUCTION

Infertility is an age-long world health concern. It affects approximately 15% of all couples with Male factor infertility contributing approximately half of these cases [1]. The drive for solutions to this problem has produced various causative findings including: environmental, dietary and genetic factors. While a host of these factors have been put forward as causes, a large number of scientists have implicated dietary life style as a main area of concern. A change in physiological status, probably due to dietary factors resulting in oxidative stress has been implicated as a potent predisposing factor in infertility [1,2].

In recent years, evidences have shown that oxidative stress may play a role in the pathogenesis of idiopathic male factor infertility, but can be reduced by consumption of antioxidant supplementation such as honey tea, coffee, vegetables, wine, sprouted grains and other food [3,4,5].

The increasing worldwide resort to coffee consumption may not be unconnected with recent findings that it contains ingredients that promote health in both male and female sexes [6]. This beverage is consumed by more than 75% of women of reproductive age in the U.S. [7]. Caffeine is the active ingredient in coffee [8] and caffeine is widely consumed in different food drinks and drugs.

A May 2012 study published in New England Journal of medicine showed that coffee drinkers who drink at least two or three cups a day were about 10% or 15% less likely to die for any reason during the 13 years of the study [9]. The history of coffee as a drink is laden with controversies. Over time, some of the world's greatest composers, thinkers and statesmen have extolled coffee's virtues while others have denounced it as a poisonous mind corrupting drug [10].

Though reports abound on the effect of coffee on human subjects especially the negative effects of coffee on maternal health particularly in the temperate regions, however, not much investigation has been carried out on the effect of coffee on Testosterone, FSH and LH in males as well as Africans generally. Not much has also been revealed as to, whether the anti-oxidant status of coffee could boost the reproductive functions of male hormones. This study wishes to investigate the interplay between the Humoral and hormonal factors on different doses of Caffeine and Coffee on the hormone levels and sperm functions in albino rats.

### 1.1 Aim of Study

The aim of this study is to determine the effects of Coffee on the reproductive functions of Wistar rats. Specifically, the study attempted to investigate:

- i. The effect of Coffee and Caffeine on general body and weights of the testis of Wistar rats.
- ii. The effect of Coffee and Caffeine on semen quality of Wistar rats.
- iii. The effect of Coffee and Caffeine on the basal levels of reproductive hormones (testosterone, FSH, LH) of Wistar rats.

## 2. METHODOLOGY

### 2.1 Research Design

One hundred and seventy-five (175) Wistar rats bred in the Animal house of the Faculty of Basic Medical Science of Delta State University were used for this study. Thirty (30) rats were used for toxicity test. Two series of pilot studies were done before commencement of the final experiments. The study proper was divided into two units; Units one and two. Seventy (70) rats used in Unit one were randomly selected into

groups of ten (10) rats of seven (7) groups each. All animals were fed with normal rat chow and water. All experimental rats were treated for four weeks period. Group 1, control, received food and water only, groups 2, 3 and 4 received 40 mg/kg, 60 mg/kg and 80 mg/kg, doses of Coffee respectively while Groups 5, 6 and 7 received 30 mg/kg, 45 mg/kg and 60 mg/kg, doses of Caffeine respectively. After administration of test solutions, animals were sacrificed by cervical dislocation and serum samples collected. The testes, liver and kidneys were also harvested for histological studies. In Unit two, Seventy-five (75) rats of both sexes were mated and treated in varying mating and treatment groups; Normal male + Normal Female (control) Coffee Treated female + Normal male Caffeine treated female + Normal male Coffee treated male + Normal female Caffeine treated male + Normal female until after gestation then, the pub weight, litter size, survival rate and gestation length were studied. The experiments in this unit (two) were done in two phases; first phase were simply treated and results taken while in second phase, treatments were stopped for withdrawal period of two weeks before allowing the experiment to continue or stop in different cases. All results were expressed as Mean  $\pm$  Standard deviation. Using the One-way Analysis of Variance (ANOVA), evaluation of data for statistical significance was carried out with the SPSS Statistical software (vaesion 20). P-value  $\leq$ 0.05 was considered statistically significant.

## 2.2 Ethical Considerations

Approval for the use of animals was granted by the Medical Ethical Committee, Delta State University, Abraka, Delta State, Nigeria.

## 2.3 Procedure

### 2.3.1 Preparation of stock solution of caffeine

#### 2.3.1.1 High dose (60 mg/kg)

1200 mg (1.2 g) of Caffeine was weighed with an electronic weighing balance and dissolved in 200 ml of distilled water. This gave stock solutions of 1200 mg/200 ml (6 mg/ml).

#### 2.3.1.2 Medium dose (45 mg/kg)

900 mg (0.9 g) of Caffeine was weighed with an electronic weighing balance and dissolved in 200 ml of distilled water. This gave stock solutions of 900 mg/200 ml (4.5 mg/ml).

#### 2.3.1.3 Low dose (30 mg/kg)

600 mg (0.6 g) of Caffeine was weighed with an electronic weighing balance and dissolved in 200 ml of distilled water. This gave stock solutions of 600 mg/200 ml (3 mg/ml).

### 2.3.2 Preparation of stock solutions of coffee

#### 2.3.2.1 Low dose (40 mg/kg)

800 mg (0.8 g) of coffee was weighed with electronic weighing balance and constituted in 200 ml of distilled water. This gave stock solutions of 800 mg/200 ml (4 mg/ml).

#### 2.3.2.2 Medium dose (60 mg/kg)

1200 mg (1.2 g) of coffee was weighed with electronic weighing balance and constituted in 200 ml of distilled water. This gave stock solutions of 1200 mg/200 ml (6 mg/ml).

#### 2.3.2.3 High dose (80 mg/kg)

1600 mg (1.6 g) of coffee was weighed with electronic weighing balance and constituted in 200 ml of distilled water. This gave stock solutions of 1600 mg/200 ml (8 mg/ml).

## 2.4 Administration of Coffee Solution

High dose (80 mg/kg), Medium dose (60 mg/kg) and low dose (40 mg/kg) were estimated from the lethal dose of coffee (192 mg/kg). For high dose, medium and low dose of coffee, 1.6 g, 1.2 g and 0.8 g were dissolved in 200 ml of distilled water making the stock concentration to be (8 mg/ml), (6 mg/ml) and (4 mg/ml) respectively.

The body weights of male Wistar rats were taken and the doses of test drugs in millilitre to be administered were calculated.

## 2.5 Administration of Caffeine Solution

Caffeine was administered to experimental animals according to their body weight, such that animal weighing 200 g, 150 g, 170 g received 2 ml, 1.5 ml and 1.7 ml respectively. Caffeine was administered orally using oral canola.

## 2.6 Sample Collection

At the end of experimental administrations, the wistar rats were anesthetized in a desiccator

containing cotton wool soaked with chloroform. After they had attained deep anesthesia, they were brought out of the desiccator and a laparotomy was carried out (by making a V-shape incision in the abdominal region with the aid of a surgical scissors) and the visceral organs were exposed. The epididymis was ruptured and semen analysis was done immediately while the testes collected into a well labeled plain bottle containing 10% formal saline awaiting histological analysis.

## 2.7 Determination of Body and Organ Weight

The body weight of the experimental male wistar rats were initially taken after procurement, (before administration). The final weights of the wistar rats were determined before sacrificing and sample collection. Percentage weight gain was later calculated using the formula:

$$\text{Percentage weight gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times \frac{100}{1}$$

The testes were harvested and trimmed of adherent tissues. They were placed in filter paper and weighed. Relative organ weights were also determined thus:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrificing (g)}} \times \frac{100}{1}$$

## 2.8 Estimation of Semen Count

Semen fluid was diluted 1 in 20. Sodium bicarbonate formalin diluting reagent was used to kill the cells to ensure proper counting. Using a Pasteur pipette, the improved Neubauer counting chamber was filled with well mixed diluted semen. It was allowed to settle for about 3-5 minutes. Using 10x objective, the number of semen was counted in an area of 2 sq mm. The number of spermatozoa in 1ml was calculated by multiplying the number counted by 100 000 (Thakkar et al. [11]).

## 2.9 Estimation of Semen Morphology

A thin smear of liquefied well mixed semen was made on a slide. While still wet, the smear was fixed with 95% ethanol for 5-10 minutes and was allowed to dry. The smear was washed with sodium bicarbonate-formalin solution to remove any mucus which may be present. The smear

was rinsed with several changes of water. The smear was covered with diluted methylene blue and allowed to dry. The proportion of normal and abnormal spermatozoa was examined using 40 x objectives (Torjesen et al. [12])

## 2.10 Hormonal Assay

### 2.10.1 Determination of follicle stimulating hormone (FSH)

**Principle:** The FSH quantitative test was based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-a.-FSH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-~FSH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies.

### 2.10.2 Determination of luteinizing hormone (LH)

**Principle:** In this test, the targeted antigen, LH, in the sample is immobilized through binding between the streptavidin coated onto the walls of the microplate and the biotin molecule that is part of the biotinylated monoclonal antibody added in the enzyme conjugate. Together with another specific enzyme-conjugated antibody, the target antigen and biotinylated antibody forms a sandwich complex on the walls of the microplate after a period of incubation (Tuck and Francis, [13]).

### 2.10.3 Determination of testosterone

**Principle:** Testosterone EIA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated weds are incubated with 10 JII of Testosterone standil'ds, controls, patient' samples, 100 J.LI Testosterone-HRP conjugate reagent and 50 JII rabbit anti- Testosterone reagent at 37°C for 90 minutes.

### 2.10.4 Determination of oestrogen

**Principle:** The E2 EIA is based on the principle of competitive binding between E2 in the test

specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-Coated wells are incubated with 25 ul E2 standard, control and sample. 100 ul Estradiol-HRP Conjugate Reagent and 50 ul rabbit anti-Estradiol reagent at room temperature (18-25°C) for 90 minutes (Tuck and Francis, [13]).

### 2.11 Statistical Analysis

The evaluation of data for significance was done, using One-way Analysis of Variance (ANOVA). The statistical data were analysed using the SPSS 20 Statistical software. A p-value of  $P < 0.05$  was considered statistically significant.

### 3. RESULTS

From Figure 1, administration of caffeine low dose caused weight gain of 4.10%. However, administration of caffeine high and medium dose caused weight reduction of -6.62% and 8.33% respectively when compared to control (with weight gain of 0.80%).

From Figure 2, there was percentage weight gain of 4.83%, 1.48% and 4.86% in high dose, medium dose and low dose respectively when compared to control group.

From Figure 3, there was significant difference ( $P \leq 0.05$ ) in relative testes weight among groups (control, high dose, medium dose and high dose) means.

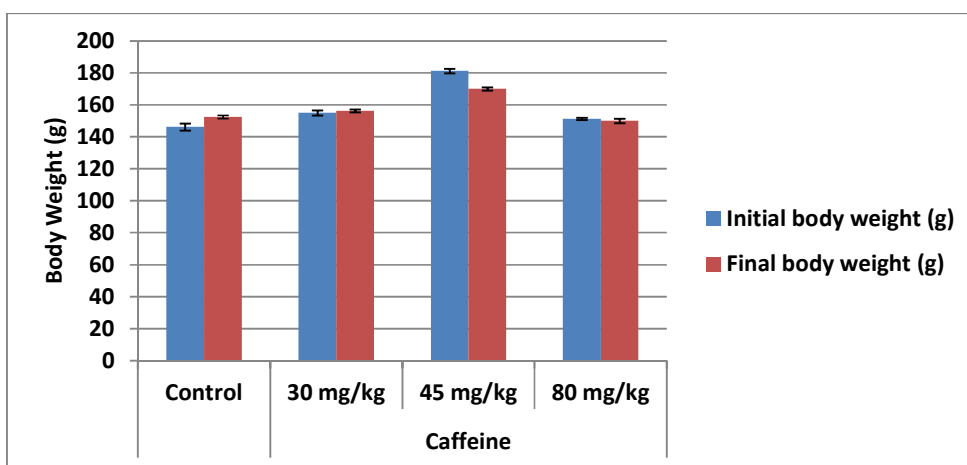


Figure 1. Body weight test carried out with respect to treatment with Caffeine

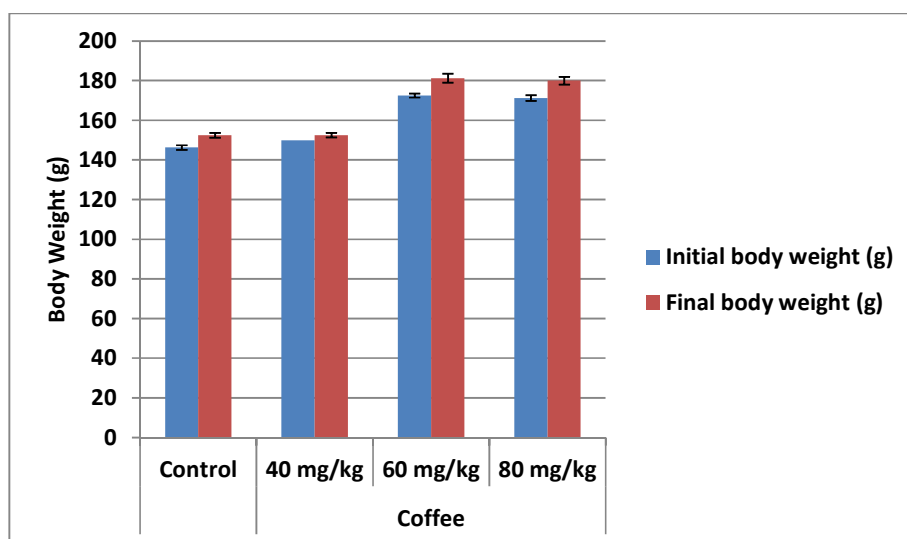


Figure 2. Body weight test carried out with respect to treatment with Coffee

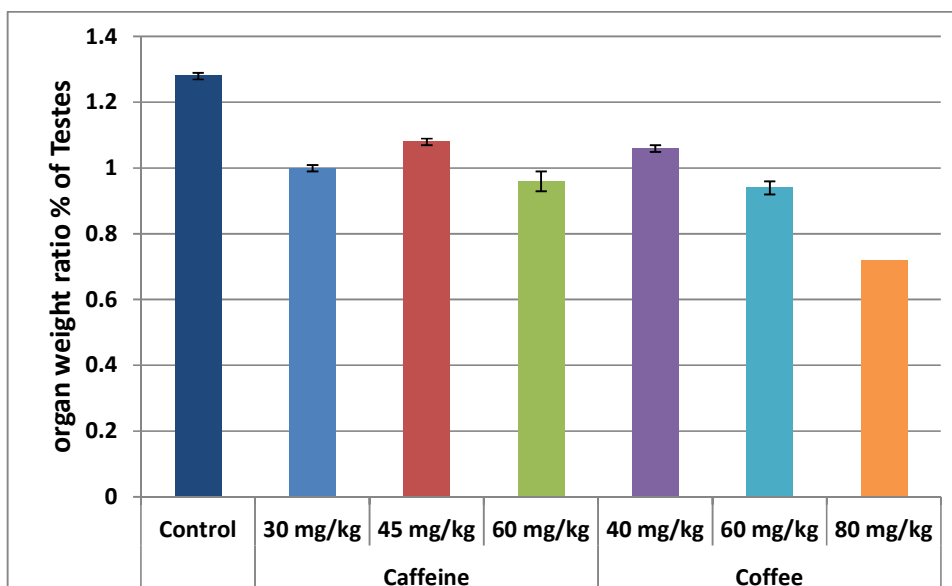


Figure 3. Showing organ weight ratio of Testis carried out with respect to treatment with Coffee and Caffeine

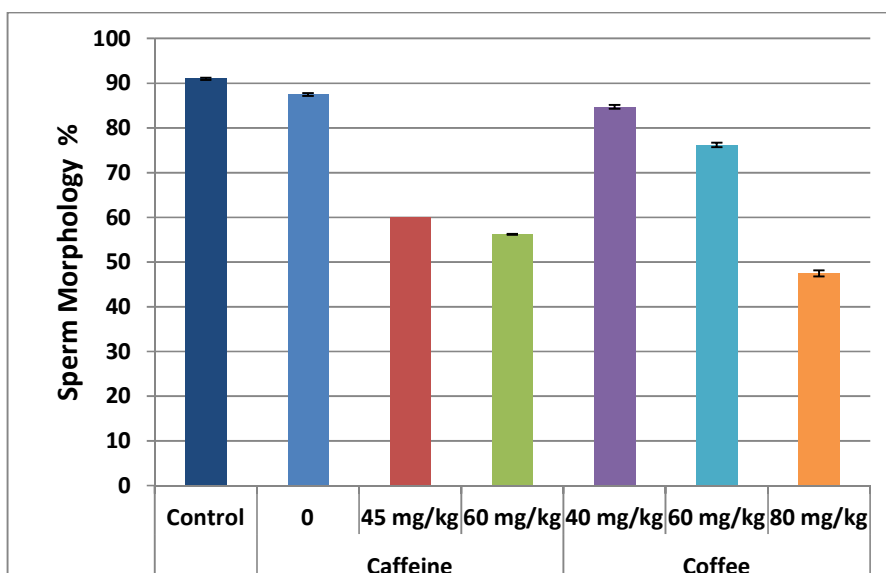


Figure 4. Results of sperm morphology carried out with respect to treatment with Coffee and Caffeine

From Figure 4, there was significant ( $P \leq 0.05$ ) decrease in semen morphology among groups only in higher doses. Lowest dose for both solutions showed a non-significant decrease.

From Figure 5, there was significant ( $P \leq 0.05$ ) decrease in semen progressive motility among groups except at lowest dose Caffeine treatment where there was non-significant decrease.

From Figure 6, there was significant decrease ( $P \leq 0.05$ ) in sperm count following the administration of Coffee and Caffeine at all doses. Values in all doses showed significantly ( $P < 0.05$ ) decrease when compared to value in control group in a dose dependent manner, i.e. The lowest values are seen in the highest doses.

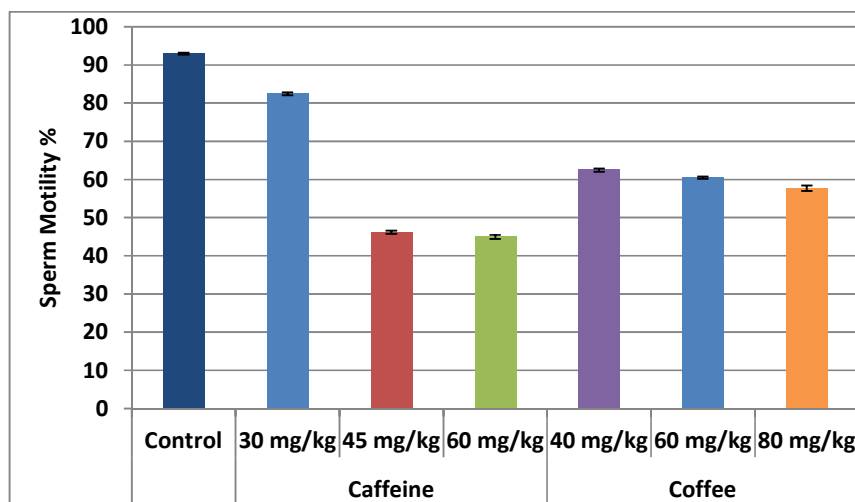


Figure 5. Results of sperm motility carried out with respect to treatment with Coffee and Caffeine

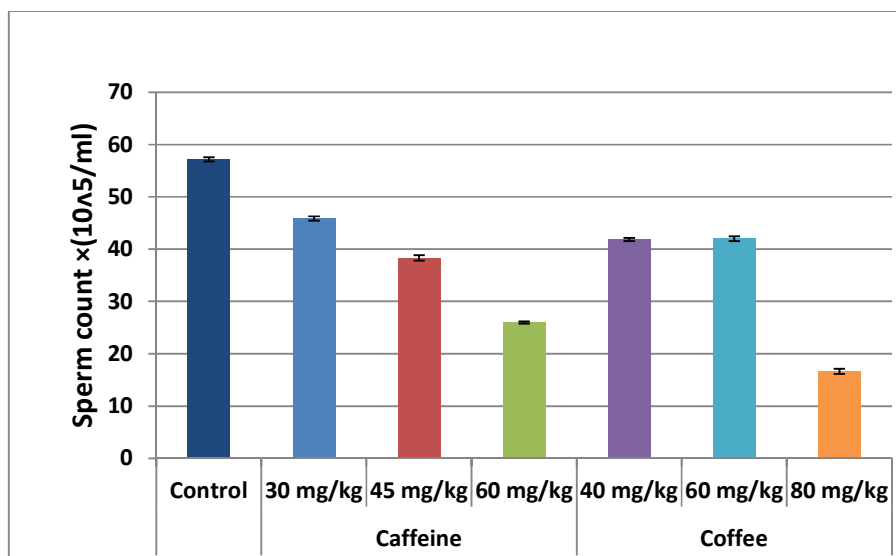


Figure 6. Results of sperm count carried out with respect to treatment with Coffee and Caffeine

From Figure 7, there was significant decrease ( $P \leq 0.05$ ) in serum Testosterone levels following the administration of caffeine and coffee at all doses. Testosterone values in all doses significantly ( $P < 0.05$ ) decreased when compared to value in control group in a dose dependent manner, i.e. The highest values are seen in the lowest doses followed by medium and highest coffee, showing dose dependence. This pattern was similar except caffeine at lowest dose treatment.

From Figure 8, there was significant decrease ( $P \leq 0.05$ ) in serum FSH levels following the administration of caffeine and coffee at all doses.

FSH values in all doses significantly ( $P < 0.05$ ) decreased when compared to value in control group in a dose dependent manner, i.e. The highest values are seen in the lowest doses followed by medium and highest coffee, showing dose dependence.

From Figure 9, there was significant decrease ( $P \leq 0.05$ ) in serum LH levels following the administration of caffeine and coffee at moderate and high doses for coffee but only high dose of Caffeine showed significant decrease. The effect is dose dependence curve but in caffeine administration there was an anomalous dose effect on LH levels.

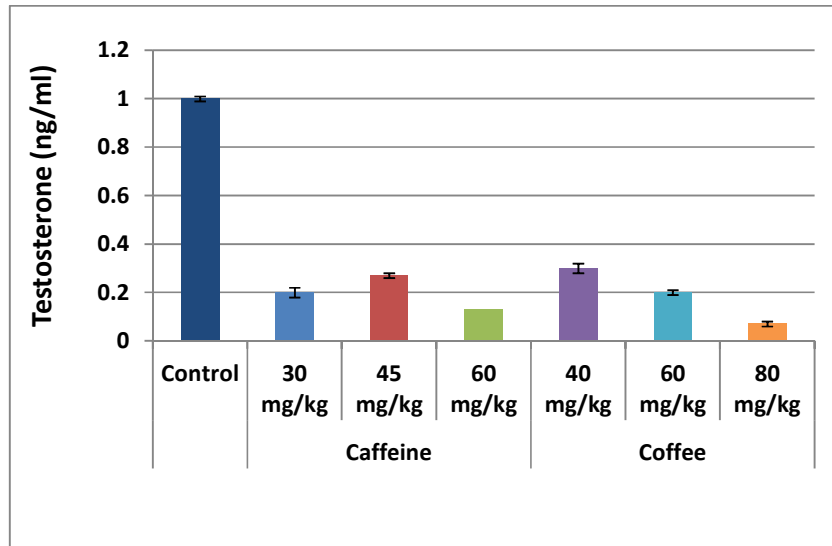


Figure 7. Results of testosterone levels carried out with respect to treatment with Coffee and Caffeine

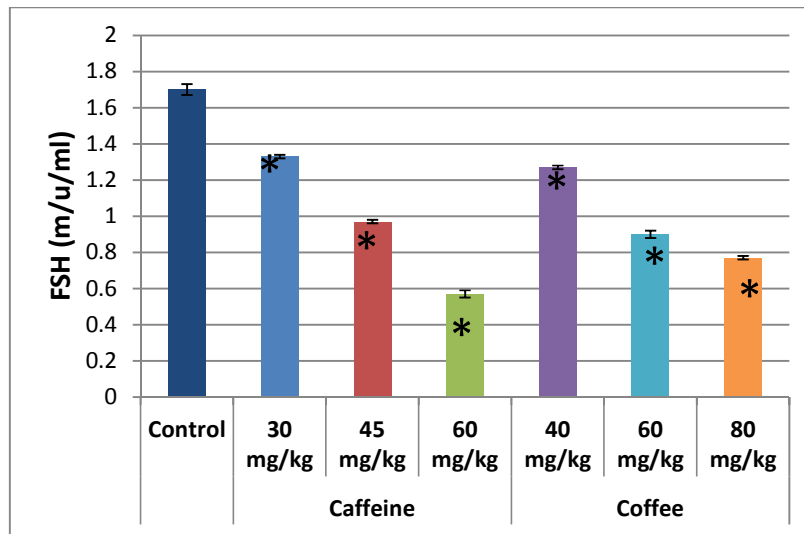


Figure 8. Showing Results of FSH levels carried out with respect to treatment with Coffee and Caffeine

#### 4. DISCUSSION

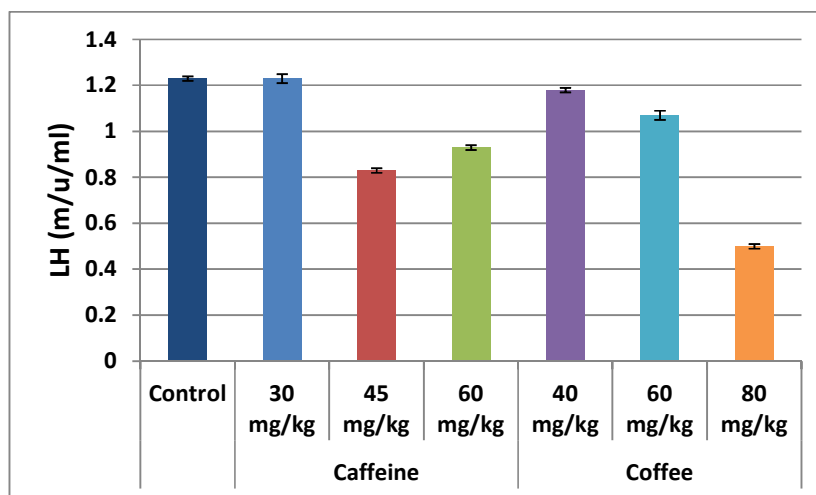
Controversies on coffee consumption are ranging, because coffee has also been found to produce some negative (undesirable) effects.

Caffeine is the World's most widely consumed psycho-active substance, but unlike most other psychoactive substances, it is legal and unregulated in nearly all jurisdictions [14,15]. An estimated 80% of the world's population

consume a caffeine-containing substance daily [16]. Given this widespread use, the potential health effects of coffee are important for public health as well as for helping an individual make an informed choice regarding coffee consumption.

In the present study, the effects of coffee consumption on various reproductive parameters were studied. The histology of testes and semen quality, testicular weight and animal body weight were evaluated.





**Figure 9. Results of LH levels carried out with respect to treatment with Coffee and Caffeine**

#### 4.1 Effect of Coffee on General and Organ Weight

The findings from this study demonstrated that consumption of coffee may have the potentials of decreasing body weight. There was no significant change in weight ( $P < 0.05$ ); indicative that weight decrease resulting from coffee treatment must have been counterbalanced by weight gain due to growth and adequate feeding over the duration of experiment. This closely agrees with previous reports that; coffee has an inverse associated with weight gain, and reduces body weight [17]. More so, findings from this study also agree with the common opinion that, significant loss in body weight could be attributed to the diuretic effect of Caffeine and its role in enhancement of fat metabolism [18].

#### 4.2 Effect on Reproductive Hormones

The results showed that coffee treatment on rats decreased both male and female hormones, though in varying proportion. A previous study to demonstrate the effects of chronic administration of caffeine and stress on feeding behaviour of rats found that caffeine elevates testosterone level [19]. This decrease could be due to effects of caffeine, probably through acting on the hypothalamic-pituitary gonadal axis.

The result also showed that administration of high dose and low dose of coffee caused significant reduction in serum testosterone when compared to control group. Testosterone is known to be critically involved in the

development of sperm cells (spermatogenesis) and therefore, derangement in testosterone level results widely from leydig cell dysfunction and testicular steroidogenic disorder [20].

The reduced level of serum LH, FSH and Testosterone could be as a result of the inhibitory effect of caffeine on the anterior pituitary gland. The anterior pituitary gland secretes FSH and LH which helps in the secretion of Testosterone and these hormones are necessary for spermatogenesis. Inhibition of the pituitary gland could lead to reduction in FSH and LH level and eventually Testosterone level [21].

Also, the result of the study showed that there was significant difference in serum FSH among high dose, medium dose and low dose following administration of coffee to wistar rats. LH level in medium dose was significantly reduced when compared to serum LH level in control group and high dose. Serum LH in low dose was also significantly decreased when compared to serum LH level in medium dose. There is no previous literature to support this finding.

Recent studies show that coffee increases blood antioxidant capacity following acute and chronic intakes, Although, all types of coffee contain relatively high concentration of antioxidant, caffeine coffee may possess even greater antioxidant activity [21,22]. Depletion of each of the antioxidant system increases the vulnerability of various tissues and cellular component of oxygen species. Moreover, other research reported that interfering with free radical metabolism with antioxidant supplement may

prevent useful activities of the body; recently, coffee has aroused scientific interest because it is rich in source of a number of phenol compounds with antioxidant effect *in vitro* [23,11,12,13].

## 5. CONCLUSION

In the various investigations carried out in the study, Coffee was seen to rival the deleterious effects of caffeine in almost all parameters measured suggesting that caffeine content of coffee is up to the level at which pure caffeine exerts its effect. Both Caffeine and Coffee treatment showed a dose- dependent effect on most parameters measured. Findings from this study established for the first time that Coffee consumption affects both male and female in the outcome of their reproductive activities and that the most dangerous period to consume this food drink is in the third trimester of gestation period.

## 6. PROSPECT FOR FURTHER STUDIES

The results from this study have necessitated recommendations for further studies to be carried out on the following:

- i. Effects of Coffee on the neuro-endocrine system (eg dopamine, noradrenaline) in the hypothalamus and other sexual behaviour regulatory centres in the brain.
- ii. An electron microscopic evaluation of any ultrastructural changes in the testes, epididymis, anterior pituitary gland and the hypothalamus.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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