

Effect of Caffeine (Nescafé) on Serum Testosterone Levels in Male Wistar Rats

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Abstract: Caffeine is one of the most frequently ingested pharmacological agents. It is contained in several beverages; with dearth of literature on its effect on reproductive parameters. This present study aimed to determine the effect of aqueous solution of caffeine on serum testosterone level in wistar rats. Forty-five male albino wistar rats weighing between 120g-200g were obtained for this study and were grouped based on their body weight into five groups of nine rats each. Group 1, served as the control and received water rats chow ad libitum while groups 2,3,4 and 5, serve as test groups and received 20mg/kg, 60mg/kg, 100mg/kg and 140mg/kg body weight doses of caffeine respectively, orally once daily for 21 days. Three animals were sacrificed from each group on the 7th, 14th and 21st day following administration. Blood samples were collected through cardiac puncture for serum testosterone analysis and the testes were harvested on the 21st day for histopathological examination. There was a dose-dependent significant ($p < 0.05$) increase in serum testosterone level on the 7th, 14th and 21st day for 100mg/kg and 140mg/kg; and a significant ($p < 0.05$) increase in 21st day for 60mg/kg between dose of caffeine when compared to the control. The histopathological examination of the testes showed normal testicular architecture; indicative of normal testicular integrity. These findings revealed that caffeine [22] may have a possible potential to stimulate spermatogenesis. Hence its consumption may lead to an increase in sex drive and improve male factor fertility.

Keywords: Caffeine, beverages, testosterone. Testes, spermatogenesis.

INTRODUCTION

Approximately 13% of the general reproductive age population is challenged with fertility problems, and male factor seem to contribute for up to 30% of them [1]. Testosterone is the male sex hormone important for sexual and reproductive development [2]. Caffeine (1,3,7 trimethylxanthine) [3] is one of the most widely used psychoactive substance due to its ubiquitous occurrence in commonly consumed beverages such as coffee, tea and cola [4]. It easily crosses biological membrane and is rapidly distributed throughout the body [5]. The caffeine molecule is easily absorbed by humans, having approximately 100% of bioavailability when taken by oral route and reaching a peak in the blood with 15-45mins after its consumption [6]. Caffeine has a number of biological effects including central nervous system stimulation, increased secretion of catecholamine, relaxation of smooth muscle and stimulation of heart rate. Whereas a moderate intake may confer a modest protective effect against some cardiovascular system disease and on the metabolism of carbohydrate and lipids including arrhythmia, diabetes, liver disease [7] and even Parkinson's disease [8], excessive amounts may lead to deleterious health effects [6]. Moreso, the increasing worldwide resort to coffee consumption may not be

unconnected with recent findings that it contains ingredients (caffeine) that promote health in both male and female sexes [9]. However, the present study was designed to evaluate the effect of caffeine in the coffee drink, NESCAFÉ, on serum levels of testosterone and on the histology of the testes of wistar rats. The results of this study will create public health awareness and promote informed choice regarding caffeine consumption.

MATERIALS AND METHODS**Study centre and period**

This study was conducted at the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria, between May and July, 2018.

CAFFEINE AND OTHER REAGENTS

Officially, according to NESCAFÉ com, their soluble coffee contains about 65mg of caffeine per 175ml (6 fl. oz.) cup. Esegbue *et al.*, [10] suggested that caffeine content of coffee is up to the level at which pure caffeine exerts its effect.

Aqueous solution of caffeine at different concentration were prepared from the coffee product-

NESCAFÉ manufactured by Nestle’ cote d’Ivoire, in the Department of Biochemistry Laboratory, University of Port Harcourt. All reagents used were of analytical grade.

EXPERIMENTAL ANIMALS

Forty-five (45) male albino wistar rats weighing 120g-200g were purchased from animal farm of Department of Human Anatomy and kept at the animal house, Department of Human Anatomy, University of Port Harcourt, Nigeria, in spacious and well ventilated cages at room temperature and under natural day/night cycles. They were allowed to acclimatize for seven days and had free access to feed and water *ad libitum*-“principles of laboratory animal care” NIH Publication No 85, revised 119 [11], were followed as well as specific national laws where applicable [11].

EXPERIMENTAL DESIGN

Forty-five (45) experimental rats were randomly grouped into five groups of nine animals each according to their body weight after the period of acclimatization. Group one: served as the control, received water and normal rat chow *ad libitum* while groups two to five served as the test groups such that Group two received 20mg/kg body weight dose of caffeine, group three received 60mg/kg body weight dose of caffeine, group four received 100mg/kg body weight dose of caffeine and group five received 140mg/kg body weight dose of caffeine, by oral administration, once daily for a period of 21 days.

At the end of each week, being the 7th, 14th and 21st day following administration of graded doses of caffeine, three animals each were sacrificed per group and on day 21 testes were harvested for histopathological examination.

The animals were sacrificed under anesthesia (diethylether suffocation) and blood samples were collected through cardiac puncture into plain sample bottles. Serum was separated at 3,000rpm for 10 minutes and stored in the refrigerator at -4^oC for subsequent hormonal analysis. The testes were collected and stored in plain bottles containing bouins fluid before embedding in paraffin wax and further processed for examination [12, 13].

HORMONAL ASSAY

Serum testosterone levels was determined using the Elisa kit [14].

STATISTICAL ANALYSIS

Data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. Data were expressed as mean ± SEM and data were analyzed using one way analysis of variance (ANOVA). Differences were considered significant at 95% confidence interval (p<0.05).

RESULTS AND DISCUSSION

The results obtained from the analysis of the effect of caffeine on body weight and serum testosterone levels are presented in Tables 1-2 and histopathology of the testes at day 21 is shown in figure 1-5.

Table-2: Effect of Aqueous solution of caffeine on body weight of wistar rats

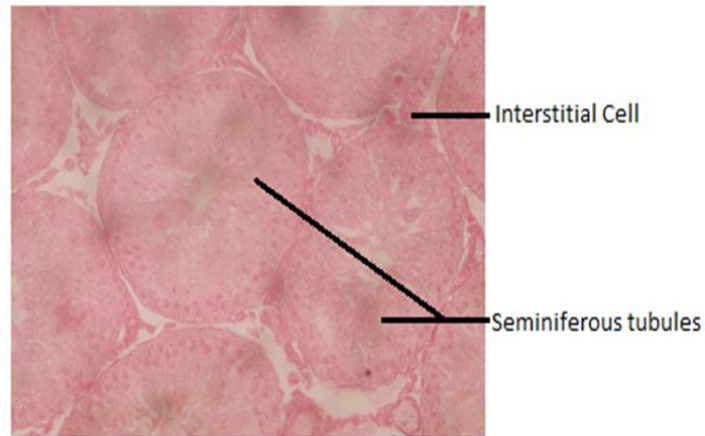
Group	Dose administered	Day 0	Day 7	Day 14	Day 21
1	Control	120.00 ± 0.00	160.00 ± 0.00	200.00 ± 0.00	173.35 ± 25.09
2	20mg/kg	120.00 ± 0.00	156.67 ± 5.77	200.00 ± 0.00*	180.00 ± 0.00*
3	60mg/kg	140.00 ± 0.00	176.67 ± 25.17	193.35 ± 11.55*	186.67 ± 11.55
4	100mg/kg	160.00 ± 0.00	193.33 ± 11.55	186.67 ± 23.09	186.67 ± 23.09
5	140mg/kg	200.00 ± 0.00	200.00 ± 0.00	196.67 ± 5.77	223.33 ± 15.28*

Values are expressed as mean ± SEM; n=3
* Significant at p<0.05 when compared with day 0

Table-2: Effect of Aqueous solution of caffeine on testosterone in wistar rats

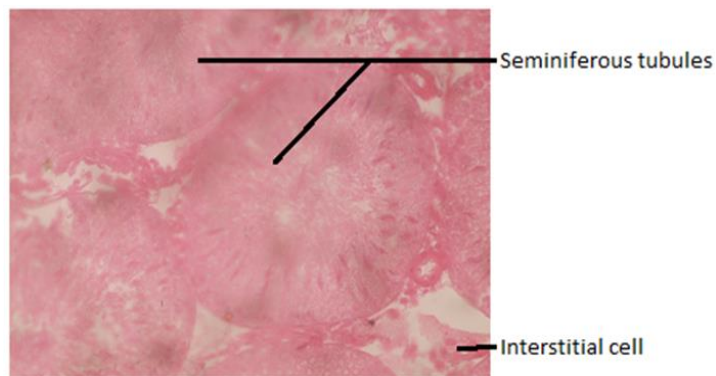
Groups	Dose administered (mg/kg)	Day 7 (ng/ml)	Time (days) Day 14 (ng/ml)	Day 21 (ng/ml)
1	Control	0.67 ± 0.33	0.60 ± 0.29	0.65 ± 0.32
2	20.00	0.96 ± 0.52	1.16 ± 0.61	1.82 ± 0.92
3	60.00	1.52 ± 0.95	1.48 ± 0.63	2.44 ± 0.31*
4	100.00	2.40 ± 0.34*	1.95 ± 0.90*	2.49 ± 1.32*
5	140.00	2.74 ± 0.38*	2.53 ± 0.41*	2.82 ± 1.16*

Values are expressed as mean ± SEM, n = 3;
*Significant at p<0.05 when compared with control



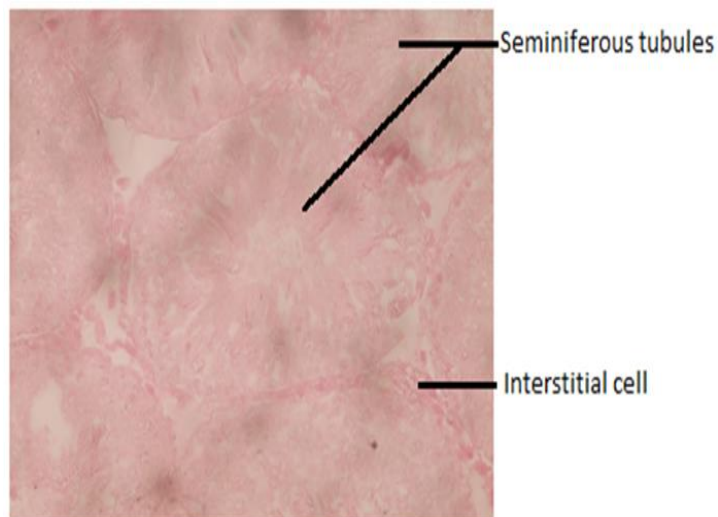
Photomicrograph of Testis Mag X 400 H&E

Fig-1: Control photomicrograph of testis at week 3 shows normal histology



Slide 15: Photomicrograph of Testis MagX400 H&E

Fig-2: Photomicrograph of testis at week 3 shows normal histology



Slide 5: Photomicrograph Testis Mag X 400 H&E

Fig-3: Photomicrograph of testis at week 3 shows normal histology

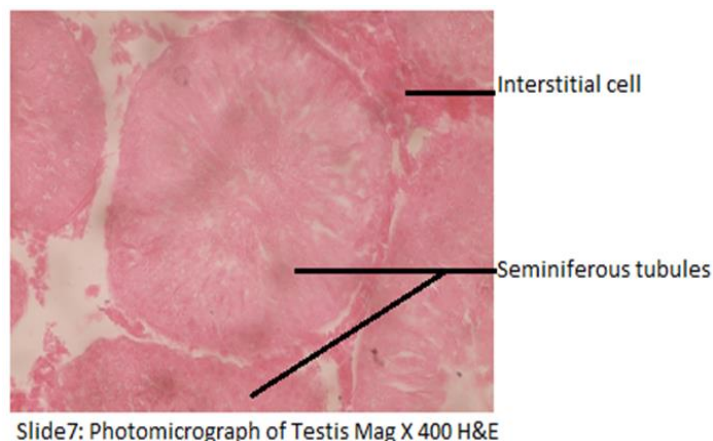


Fig-4: Photomicrograph of testis at week 3 shows normal histology

The present study investigated the effect of caffeine (NESCAFÉ) on serum testosterone levels and histopathology of the testes using wistar rats as models and body weight of the animals were determined weekly as a general index of overall health.

Table 1 indicates that the administration of 20mg/kg, 60mg/kg and 140mg/kg body weight dose of caffeine produced a significant increase in body weight at longer duration of exposure i.e. day 14 and 21 when compared to day zero; however no significant increase in body weight was obtained for the control group and group three (3) which were administered 100mg/kg body weight dose of caffeine. This findings suggests that consumption of caffeine may have the potential of increasing body weight at the doses considered or could be indicative of weight gain due to growth and adequate feeding over the duration of the experiment, this closely agrees with a previous study, that there is no effect of coffee on dietary intake and appetite related feeding in normal weight individuals [15]. However this result is not consistent with Zheng, *et al.* [16].

Table 2 shows the effect of aqueous solution of caffeine on serum level of testosterone in wistar rats. There was a significant increase ($p < 0.05$) in serum level of testosterone for 100mg/kg body weight and 140mg/kg body weight doses of caffeine following administration to day 7 when compared to the control. Also, a significant increase was obtained on day 14 at 100mg/kg body weight and 140mg/kg body weight dose of caffeine when compared with the control. However non-significant ($p < 0.05$) increase were recorded for 20mg/kg between dose of caffeine on day 7 and 14 when compared to the control. This non-significant increase is indicative of caffeine's no or mild effect at lower doses [17].

Meanwhile, on day 21, a significant dose and duration dependent increase of serum level of testosterone was obtained for 60mg/kg bw, 100mg/kg bw and 140mg/kg bw doses of caffeine when compared with the control.

A previous study revealed that male coffee/caffeine consumption has been associated with high levels of testosterone and sex hormone binding globulin (SHGB) [18] in addition [19] and Dias, *et al.* [20] stated that in both fetal and adult life, caffeine can act indirectly by impacting the hypothalamo-pituitary-gonadal system or by a direct toxic effect on the germinative epithelium, these studies explains the result from table 2 which gives an indication that caffeine can cause a pronounced change in the rate of production of testosterone, therefore suggesting the non-toxic nature but rather stimulating effect of aqueous solution of caffeine on testosterone producing cells (ley dig cells) in a dose dependent manner.

The results of the histopathological examination of the testes in figure 1-5 showed normal testicular architecture and integrity following administration of the considered doses of caffeine at longer duration. This is depicted by the interstitial spaces of the testes containing interstitial cells of ley dig and seminiferous tubules containing sertoli cells and spermatogenic cells with its distinctive lumen. However this result is supported by a previous study which stated that testosterone is known to be critically involved in the development of sperm cells (spermatogenesis) and therefore derangement in testosterone level results widely from ley dig cell dysfunction and testicular steroidegemic disorder [21-23].

CONCLUSION

The finding from this study showed that caffeine (NESCAFÉ) exert no deleterious effect on the testes and no reductive effect on serum level of testosterone hence its consumption may improve male fertility factor and hence sex drive.

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