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COMPARATIVE CHANGES IN BODY AND RELATIVE ORGAN WEIGHTS OF WISTAR RATS FED WITH COFFEE AND CAFFEINE

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ABSTRACT

Caffeine Active ingredient in coffee is an alkaloid ($C_8H_{10}O_2N_4 \cdot H_2O$) found in tea, cacao, and numerous other beverages. Overtime, caffeine has been shown increase the blood pressure, stimulate the central nervous system, promote urine formation, plus stimulate the action of the heart and lungs. However, controversies exist as to its effect on the body weight, relative to those of selected organs. Current study hitherto investigated the comparative effect(s) of coffee and caffeine on the body and relative organ weights of wistar rats. Seventy (70) adult male rats of between 140 – 250 grams were procured and randomly grouped into seven (7) of ten (10) rats each. All group A rats (Control) were fed with normal rat chow and water *ad libitum*; whereas, Experimental rats (groups B through G) received 40mg/kg, 60mg/kg and 80mg/kg doses of Coffee; and 30mg/kg, 45mg/kg and 60mg/kg doses of Caffeine for four (4) weeks respectively. After period of administration of test substances, the rats were weighed on weekly basis, sacrificed via cervical dislocation and selected visceral (Kidney, Liver, Heart and Testes) harvested (weekly) to ascertain the effect of duration and dose changes on their body and relative organ weights. Following statistical analysis (Using the one way analysis of variance – ANOVA and Student t-test), study found a significant difference ($p < 0.05$) in relative heart weight among groups (control, high dose, medium dose and high dose) except in low dose caffeine. There was also a significant ($p < 0.05$) increase in relative liver and testicular weight for medium and high doses of Caffeine but only in high dose of coffee treatment when compared to control and high dose respectively. This proved however insignificant for the kidney. Other than kidney, coffee and caffeine consumption therefore caused a dose and/or duration dependent effect on body and relative organ weights in wistar rats. Further studies aimed at corroborating the findings from this work are highly recommended.

KEYWORDS: Body Weight, Organ Weight, Coffee, Caffeine.

INTRODUCTION

One of the major ingredients in coffee is Caffeine. Caffeine (1, 3, 7-trimethyl-xanthine) is the alkaloid most present in green and roasted coffee beans; constituting between 1.0% and 2.5% by weight of dry green coffee beans which does not change during maturation of green coffee beans.[1-3] Lower concentrations of theophylline, theobromine, paraxanthine, liberine, and methylliberine can also be found in coffee. The concentration of theophylline, an alkaloid noted for its presence in green tea, is reduced during the roasting process, usually about 15 minutes at 230 °C (446 °F), whereas the concentration of most other alkaloids are not changed,[4] The solubility of caffeine in water increases with temperature and with the addition of chlorogenic acids, citric acid, or tartaric acid, all of which are present in green coffee beans. The xanthine alkaloids are odourless, but have a bitter taste in water, which is masked by organic acids present in green coffee, however.

Coffee has been demonstrated to have other beneficial effects including weight reduction, prevention of development of diabetes and hepatic fibrosis in non-alcoholic fatty liver disease and chronic hepatitis C and/ or B. One large, retrospective survey of patients who participated in a non- alcoholic fatty liver disease prevalence study, found that caffeinated coffee intake was associated with a significant reduction in fibrosis among biopsy-proven non-alcoholic steatohepatitis patients.[5] Similarly, a meta- analysis with pooled data from 457,922 participants demonstrated an inverse association between coffee and risk of diabetes.[6]

An Italian case control study, also found that, patients with decompensated cirrhosis were less likely to drink coffee than matched control and a Norwega in registry study reported that coffee consumption was associated with a lower risk of death from complications of cirrhosis.[7]

Several species of shrub of the genus *Coffea* produce the berries from which coffee is extracted. All coffee plants are classified in the large family Rubiaceae. The two main species commercially cultivated are *Coffea canephora* (predominantly a form known as 'robusta') and *Coffea Arabica*.[8]

Coffea arabica, the most highly regarded species, is native to the southwestern highlands of Ethiopia and the Boma Plateau in southeastern Sudan and possibly Mount Marsabit in northern Kenya.[9] *Coffea canephora* is native to western and central Subsaharan Africa, from Guinea to the Uganda and southern Sudan.[10] Less popular species are *Coffea liberica*, *Coffea stenophylla*, *Coffea mauritiana*, and *Coffea racemosa*.

Coffea arabica is responsible for approximately 70% of the global coffee market and *Coffea canephora* account for the rest 30%.*Coffea arabica* and *Coffea canephora* are different in many ways including their idea of growing, climate, physical aspects, chemical composition, flavour, quality and health promoting action.[11]

Various research interests have been stimulated by these unbelievable health benefits, however this has not stimulated interest in the consumption of this food drink as coffee had been known to cause more harm than good in the past. The volume of contradicting information about the health benefits of this processed plant is intriguing and perhaps another major research problem is the concentration i.e. quantity required to cause the beneficial or dangerous effects in the human body.

Aim of Study

The aim of this study was to investigate the comparative effect(s) of coffee and caffeine on the body and relative organ weights of wistar rats. Specifically, study;

1. Examined the effect of Coffee and Caffeine on general body and weights
2. Determined the changes due to dose and/or durational administration of Coffee and Caffeine on Testicular weight
3. Determined the changes due to dose and/or durational administration of Coffee and Caffeine on Heart weight
4. Determined the changes due to dose and/or durational administration of Coffee and Caffeine on Liver and Kidney weights

MATERIALS AND METHOD

Study Design

Study adopted the experimental type of research design; and comprised of control and experimental groups. Seventy (70) adult male rats were randomly selected into seven (7) groups of ten (10) rats each. All group A rats (Control) were fed with normal rat chow and water *ad libitum*. Experimental rats were treated for four (4) weeks with groups B through G receiving 40mg/kg, 60mg/kg and 80mg/kg doses of Coffee; and 30mg/kg, 45mg/kg and 60mg/kg doses of Caffeine respectively.

Location of Study

Study was conducted in the Animal holding of the college of health sciences, Delta State University, Abraka, Delta State, Nigeria.

Procurement and Preparation of Animals

Seventy (70) adult wistar rats (male only) of approximately the same age and an average body weight of between 140–250g were procured from the animal house of the Ambrose Alli University, Ekpoma, Edo State. The animals were transported and kept under a 12:12 hour light-dark cycle at room temperature in the animal house of the college of health sciences, Delta State University, Abraka, Delta State, Nigeria; following which they were allowed to acclimatize for two weeks before commencement of experiment. All animals were housed in standard cages in a clean and neat surrounding with *ad libitum* access to water and standard rat diet. Animal handling was performed with regard to CPCSEA guidelines, and the University's research ethics.

Preparation of stock solution of Caffeine

High dose (60mg/kg)

About 1200mg (1.2g), 900mg (0.9g) and 600mg (0.6g) of Caffeine was procured, weighed (with an electronic weighing balance) and dissolved in 200ml of distilled water to form a stock solutions of high [1200mg/200ml (6mg/ml)], medium [900mg/200ml (4.5mg/ml)] and Low [600mg/200ml (3mg/ml)] doses of caffeine for subsequent administration to animals respectively.

Preparation of Stock Solutions of Coffee

Low dose (40mg/kg)

Similar to Caffeine standardization, About 800mg (0.8g), 1200mg (1.2g) and 1600mg (1.6g) of Coffee was procured, weighed (with an electronic weighing balance) and dissolved in 200ml of distilled water to form a stock solutions of Low [800mg/200ml (4mg/ml)], Medium [1200mg/200ml (6mg/ml)] and High [1600mg/200ml (8mg/ml)] doses of Coffee for subsequent administration to animals respectively.

Administration of Coffee Solution

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

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 [Heart, Liver, Kidney and Testes] were then exposed and harvested and weighed from each group.

Administration of Caffeine Solution

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

Determination of Body and Organ Weight

The body weights of the experimental animals were initially taken after procurement, and weekly 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:

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

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

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

Ethical Considerations

Ethical Approval for the use of animals was obtained from the Research and Ethics Committee of the College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria. Global best practices and guidelines for the use and handling of laboratory animals were also strictly adhered to.

Analyses of Data

Obtained results were presented as mean ± Standard Deviation (SD) of sample size. Mean values within and between groups were compared statistically by one way analysis of variance (ANOVA) and Student t-test. Statistical package for social sciences (SPSS version 20) was used to automate data analysis. p-value < 0.05 was considered to be statistically significant.

RESULTS

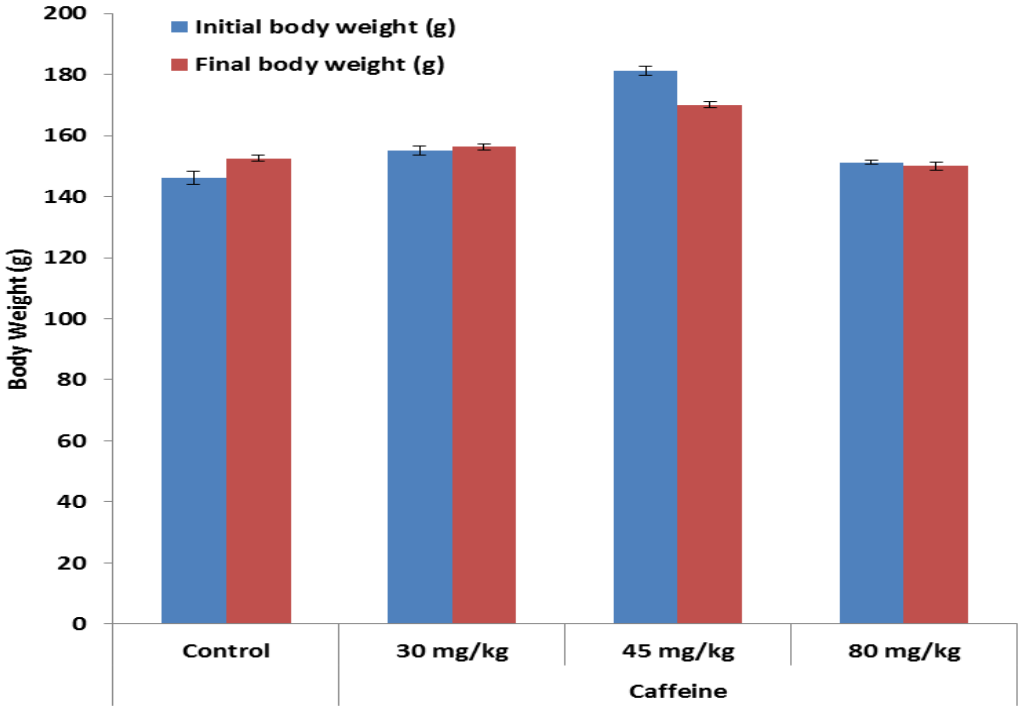

Figure I: Comparative changes in mean initial and final body weights of Caffeine Administration of Wistar Rats.

From Figure I above, 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%).

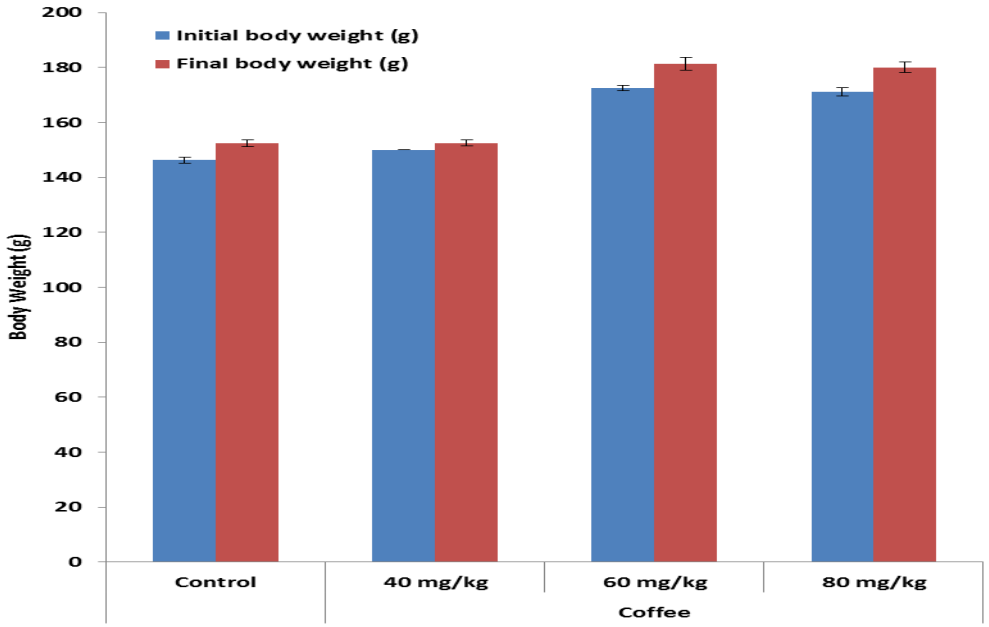

Figure II: Comparative changes in mean initial and final body weights of Coffee Administration of Wistar Rats.

From Figure II above, 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 (0.80%).

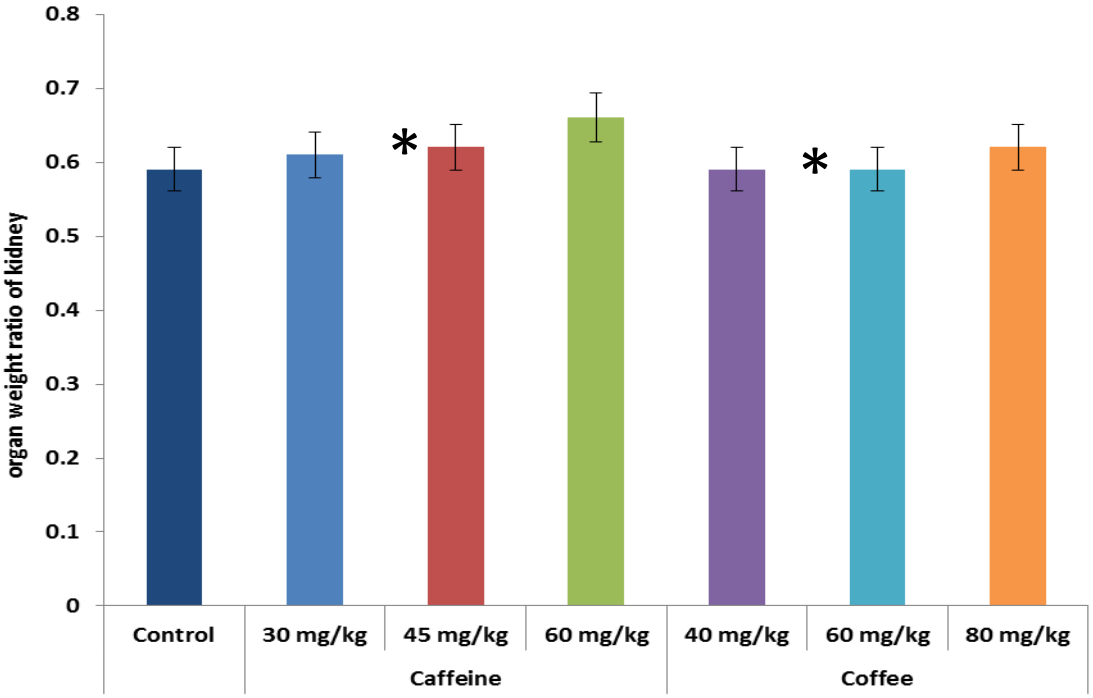

Figure III: Comparative changes in Body/Organ Weight Ratio of Kidney in Coffee and Caffeine Administration to Wistar Rats

Effect of Caffeine on relative kidney weight: From Figure III above, there was no significant difference ($P < 0.05$) in relative kidney weight among groups (control, high dose, medium dose and high dose) in the caffeine group.

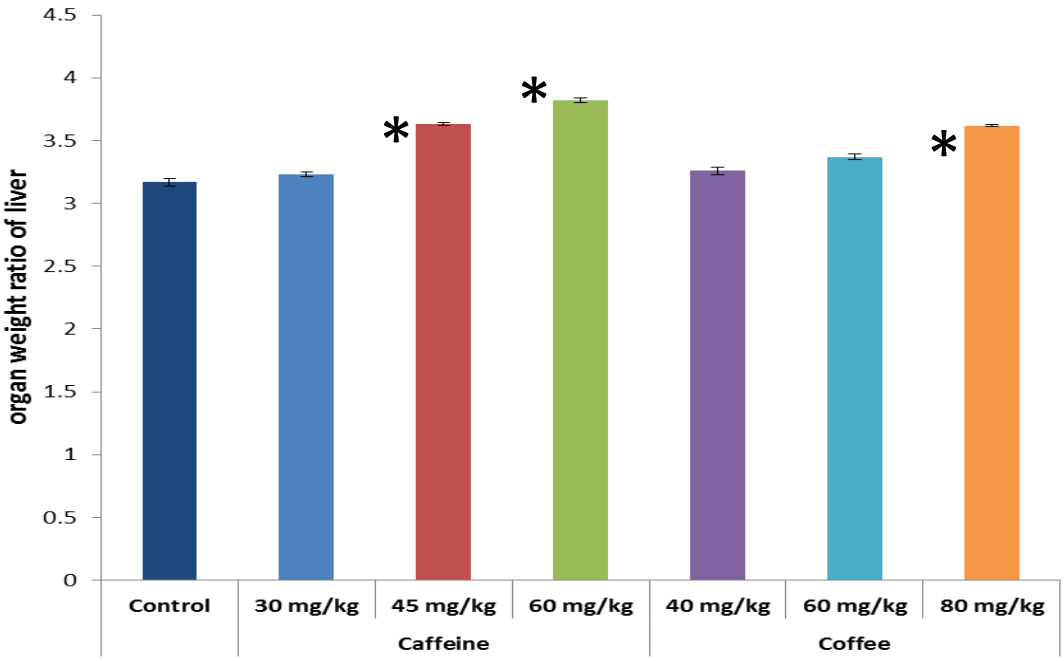

Figure IV: Comparative changes in Body/Organ Weight Ratio of the Liver in Coffee and Caffeine Administration to Wistar Rats

From Fig. 4.4 above, there was significant ($p < 0.05$) increase in relative liver weight in medium dose and high doses of Caffeine but only in high dose of coffee treatment when compared to control and high dose respectively.

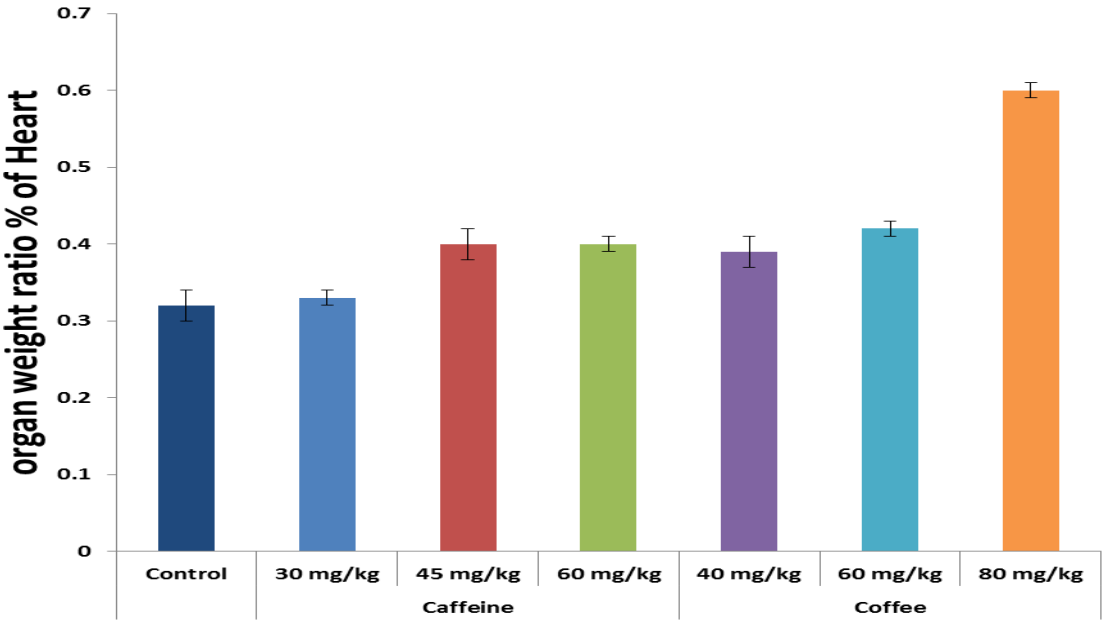

Figure V: Comparative changes in Body/Organ Weight Ratio of the Heart in Coffee and Caffeine Administration to Wistar Rats.

From Figure V above, there was significant difference ($p < 0.05$) in relative heart weight among groups (control, high dose, medium dose and high dose) except in low dose caffeine.

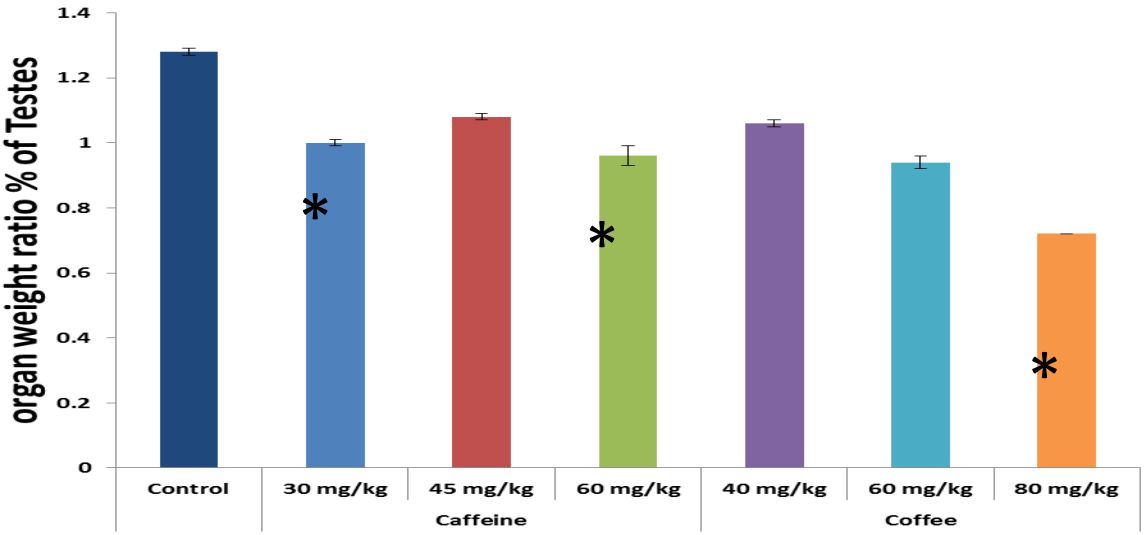

Figure VI: Comparative changes in Body/Organ Weight Ratio of the Testes in Coffee and Caffeine Administration to Wistar Rats.

From Figure VI above, there was significant difference ($p < 0.05$) in relative testes weight among groups (control, high dose, medium dose and high dose) means.

DISCUSSION

In this study, the effects of coffee and/or caffeine consumption on general body weight and those of selected organ relative to the general body weight was investigated in wistar rats fed with coffee and caffeine.

The findings from this study demonstrated that consumption of coffee may have the potentials of decreasing body weight. The was no significant change in weight ($P< 0.05$), showing that the weight decreased due to treatment must have been counterbalanced by weight gain due to growth and adequate feeding over the duration of experiment. This closely agrees with Greenberg *et al.*, (2005).[12] that reported that coffee reduces body weight and also with Lopez-Garcia *et al.*, (2006) that reported that increase in the intakes of coffee were inversely associated with weight gain.[13,15] More so this agreed with Margriet *et al*., (2005) who opined that the significant loss in body weight could be attributed to the diuretic effect of Caffeine and its role in enhancing fat metabolism. (Meri *et al*., 2004). The findings observed may indicate that the effect of coffee could be due to compounds other than caffeine."Chlorogenic acid in coffee is able to attenuate glucose absorption in the digestive track, which could help control weight" as reported by Albert *et al*., (2009).[15]

Results showed a decrease in the relative weight of testes for low, medium and high doses. There was significant reduction in the relative weight of testes of both low and medium dose when compared to control.

The finding suggests that coffee decreases testicular weight from this work. This is in agreement with Zhengwi et al., (1998) work on heterogeneous pattern of spermatogenic impairment and Releigh et al., (2004) work on stereological analysis of human testes stated that coffee decreases testicular weight. This could be as a result of the reduction of the sizes of the seminiferous tubules. A decrease in testicular weight is a biomarker of damages to the reproductive organs.

Studies have shown that dietary caffeine (0.1%) has detrimental effect on spermatogenesis and ejaculatory response of roosters (Bray et al., 1974) and scattering vascular degeneration of spermatogenic cells in rat testes (0.5%) (Gan et al., 1994).[16] More also, results seen in figure V shows significant ($P< 0.05$) reduction in relative testes weight of medium dose and low dose when compared to control. Relative testes weight of medium dose was significantly ($P< 0.05$) lower than that of high dose. This is in agreement with Yoshida et al., (2002) that coffee decreases testicular weight.[17] A decrease in testicular weight is a biomarker of damage to the reproductive organs.

CONCLUSION

Being one of the world's most popular beverages, the capability of coffee and caffeine in offsetting various health indicators across body systems has been reported. In this study however, the effect of coffee/caffeine consumptions on general body weight and those of selected organ relative to the general body weight was investigated in wistar rats. The dietary intake of moderate quantities of Coffee in this study significantly increased altered body and relative organ weights of the liver, heart and testes; causing an insignificant increase in kidney on the contrary. Albeit; this effect spans from high, medium and low doses of coffee and caffeine consumption in experimental rats, to duration-dependent changes over a period of time (4 weeks).

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