Comparative Effects of *Garcinia kola* and Coffee Diets on Learning and Memory in Mice

A. U. Nmaju[1], S. A. Bisong[2*], A. A. Nwankwo[1], I. E. Joshua[2] and E. E. Osim[2]

[1]*Department of Physiology, Faculty of Medicine and Health Sciences, Abia State University Uturu, Nigeria.*
[2]*Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors EEO and AUN designed the study, authors AUN, IEJ and AAN performed the laboratory work (phytochemistry and behavioural experiments) while author SAB performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EEO and SAB managed the analyses of the study, while authors SAB and AUN managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: *Garcinia kola* (bitter kola) is one of the most common masticatories in Nigeria having social and ethnomedicinal applications. *G. kola* seeds are believed to contain caffeine, one of the major constituent of coffee which is also believed to improve memory.
Aim: This study was set to ascertain the caffeine content of *G. kola* and compare the effects of *G. kola* and coffee diets on learning and memory in mice.
Methodology: Thirty male CD1 mice were randomly assigned into three groups, viz; control, *Garcinia kola diet* (30%w/w) and coffee diet (2%w/w) groups. Chemical content and LD_{50} of the *Garcinia kola* and coffee were determined using standard methods. Daily food intake, water intake and body weight changes were also measured for 31 days before testing for learning and memory. The Morris water maze was used to assess learning and memory.
Results: The major constituents of *Garcinia kola* were alkaloids (high quantities),

_____

*Corresponding author: Email: bisongsa@yahoo.com;

saponins, tannins, flavonoids, glycosides, sterols and phenols found in moderate quantities. The coffee contained high quantity caffeine (4.7%) whereas alkaloids, saponins, tannins, flavonoids, and phenols were present in slight quantities. Coffee diet decreased food intake, water intake body weight change in the mice when compared to *G. kola* and control diet groups (p<0.05). On the other hand, coffee diet fed mice showed better learning and memory when compared to *G. kola* diet-fed and control mice. *Garcinia kola* diet did not affect learning and memory.
Conclusion: Coffee diet decreased food and water intake and body weight, but improved learning and memory in mice when compared to *Garcinia kola.*

1. INTRODUCTION

Garcinia kola (bitter kola) is a species of plant used traditionally for many purposes in Africa. The existing belief that *G. kola* contains caffeine, which is the most active ingredient in coffee [1], stimulated our interest in comparing the effect of *Garcinia kola* and coffee on learning and memory*,* bearing in mind that both bitter kola and coffee are widely consumed as stimulants[2].

Garcinia kola belongs to a family of tropical plants known as *Guttiferae* [3]. The seeds of this plant are used in African traditional medicine for various therapeutic purposes based on pharmacological effects of the active components (flavonoids) in the seed and other parts of the plants [4]. The therapeutic potentials of *Garcinia kola* and its use in African traditional medicine cannot be overemphasized [5]. The seed extracts have been shown to possess anti-inflammatory [6], anti-diabetic [7], antimicrobial [6,8], anti-hepatotoxic, inhibition of drug metabolism and molluscicidal activity [9,10]. The ability of *G. kola* to boost immunity among its other pharmacological activities is believed to be related to its antioxidative activity [6]. The seeds of *G. kola* have also been used traditionally to treat hepatitis and other viral infections such as those caused by influenza and Ebola viruses. It has also been used as antidote for ingested poison and for oral hygiene [6].

Coffee is a common beverage that is made by brewing the roasted coffee beans from the coffee plant. The most active ingredient of coffee is caffeine. Caffeine, a xanthine alkaloid present in many common beverages, is among the world`s most extensively consumed psychoactive substance. It has weak reinforcing property inducing self-administration both in human and non-human primates [11].

Moderate doses of coffee have a stimulant effect on the central nervous system (CNS) thus increasing alertness. However, at high doses caffeine can suppress activity. Caffeine also has a stimulant effect on the heart and respiratory system, enhances mood and boosts energy release. Besides the stimulant effect of caffeine, which causes increased alertness, it also decreases food and water intake, decreases body weight and improves learning and memory [12].

Garcinia kola is readily available and freely consumed in traditional ceremonies in many part of the Nigeria. Just like coffee, many locals consume it to stay awake and brighten their mood. It is generally believed, although it has not been proven, that *Garcinia kola* keeps people awake and can sustain muscle tone. It is also believed that *G. kola* contains caffeine and therefore may also affect learning and memory.Therefore, the aim of this research was

to compare the effect of *G. kola* diet and coffee diet on learning and memory, after confirming the presence or otherwise of caffeine in either diets by phytochemical analysis.

2. MATERIALS AND METHODS

2.1 Preparation of *Garcinia kola* (Bitter Kola) and Coffee Diets

Fresh seeds of *Garcinia kola* were obtained from the Okigwe market in South-eastern Nigeria in a large quantity. The seeds were washed in water to remove debris, allowed to dry and then the hulls of the seed removed. The seeds were then cut to smaller pieces, sun dried to a constant weight and then ground to fine powder which was safely stored until required for use. Thirty grams powdered *G. kola* was mixed with 70g of normal rodent chow or feed and both were blended together using electric blender. This gave 30%w/w *Garcinia kola* diet [13].

Coffee (Nescafé) was purchased from a provisions store in Okigwe in South-eastern Nigeria. Two grams of powdered coffee was blended with 98g of normal rodent chow to form a2%(w/w) coffee diet. Every 100g Nescafe contained 4.72g of caffeine. Therefore, 2g coffee (Nescafe)contained 0.0944g caffeine or 94.4mg caffeine. So, 0.0944g of caffeine was mixed with every100g of normal rodent chow. Therefore, the coffee diet comprised approximately 0.1% caffeine.

2.2 Lethality Study for *Garcinia kola* and Coffee (Nescafe)

Graded percentages of *G.kola* diet (10%, 20, 30, to 70% *G. kola* diet) were fed to seven groups of mice (n = 6 per group).All of these diets did not show any lethality. The median dose/ diet, 30% *Garcinia kola* diet (30g of *G. kola* mixed with every 70g of rodent chow) was considered safe for the animals and so was given to the test animals. Similarly, graded percentages of coffee diet (0.5% to 10% of coffee diet) were fed to 7 groups of mice (n=6 mice per group) but no deaths were recorded. So, the 2% coffee diet (2gm of coffee mixed with every 98gm of rodent chow) given to the test animals was considered safe for the animals.

In order to further investigate the LD50 of *G. kola* and coffee, an ethanol extract was obtained by Soxhlet extraction as described by Kumar et al. [14], using 99% ethanol as solvent. The dried *G. kola* seeds were ground to powder and placed in a cellulose thimble in the extraction chamber (of the Soxhlet apparatus), which is placed on top of a collecting flask beneath a reflux condenser. Ethanol was added to the flask, and the set-up is heated under reflux. The condensed solvent accumulated in the thimble, was siphoned into the flask beneath. This was also done for the coffee bought from a local shop to get ethanol extracts of *G. kola* and coffee respectively.

Graded doses of these ethanol extracts of *G. kola* and coffee were administered orally in another two sets of mice respectively. Probit kills within 24 hours were obtained and plotted against log dose concentration. The LD_{50} was calculated using probit kill of the dose according to the method of Lorke [15].

2.3Animal Treatment

Mice of CD 1 strain aged between 60 – 80 days oldwere kept in well ventilated space under room temperature ($25\pm$ 2°C) and 12/12 hours light/dark cycle, and allowed two weeks for acclimatization to the research environment before testing. Mice were house singly in metabolic cages where food and water intake were monitored. Each mouse in each of the three groups received 15g daily of either normal rodent feed (control), 30% *Garcinia kola* diet (*G. kola* group)or 2% coffee diet (coffee group). Each mouse was also given 15 ml of drinking water ad-libitum. After every 24 hours, the amount of food and water left was subtracted from the initial amounts given to obtain daily food and water intake per mouse. This treatment was done for 31 days. Their beddings, feed and water were hygienically handled and changed every 1 – 3 pm daily throughout the period of this treatment (31days). Body weights of the animals were also taken every 3 day interval.

2.4Phytochemistry

Determination of alkaloid content was done according to the slightly modified methods of Harborne [16] and Obadoni and Ochuko [17]. Determination of flavonoids was done using the methods of Obadon and Ochuko[17]. The presence of tannins was determination by the method of Van-Burden and Robinson [18]. Caffeine was detected on thin layer chromatography (TLC) plates in comparison with standard caffeine, and High performance liquid chromatography (HPLC) analyses were used to confirm the purity and characterization of the extracted caffeine [19].

2.5 Morris Water Maze Set-up

The Morris Water Maze developed by Richard Morris [20] for assessing visuo-spatial learning and memory was used in the study. The water maze made of a circular polypropylene pool that measured 85cm diameter and 20cm in depth was used. The pool was filled to depth of 14cm with room-temperature tap water. The water made opaque with the addition of milk to ensure camouflage of the white escape platform. The platform was submerged to about 1cm below the water surface.

The pool was then divided into four quadrants: Northwest, Northeast, Southwest and Southeast. Boundaries of this quadrant were marked on the edge of the point with masking tape and labeled, North, South, East and West. The level of water in the pool was adjusted to 1cm above the platform thus creating an invisible platform.

The pool was located in the laboratory room. On the walls of the room were mounted several posters to act as visual cues. They were also furniture and electronic (TV Set) that provided visual cues. During testing, the room was dimly lit with diffuse white light. The performance of the animals in the maze was recorded both manually and electronically, using a camcorder and behavior rescored manually afterwards.

Testing in the Morris Water Maze lasted eight days. The first three days were acquisition training with an invisible platform. The next three days were reversal training with the hidden platform in an opposite quadrant. On the seventh day a probe trial was conducted with no escape platform. On day eight, 4 trials were conducted with a visible platform.

During the test period, the mouse was placed in a clean empty cage (with paper tower bedding to allow the mice to dry more quickly) after each trial. Mice were then run in squads of 7 with 10 minutes between each trial for each mouse.

During acquisition and reversal training, as well as the visible platform task, mice were given 4 trials of 60 seconds each to locate the hidden platform (or visible platform in the visible platform task). After this, if they did not locate the platform, they were guided to the position of the platform and given 10 seconds to explore. If the mice located the platform within the 60 second, the timer was stopped and the time it took the mice to locate the platform was recorded as the swim latency.

A probe trial was conducted on day 7. At this time, there was no escape platform in the maze. Each mouse completed one trial of 60 seconds. Each mouse was placed in the maze from one of the four possible positions and allowed to explore the pool. The durations in each quadrant and the frequency of entry into the acquisition and reversal quadrants were recorded.

2.6 Statistical Analysis

Data collected during the study were expressed as mean ± SEM. Analysis of variance (ANOVA) and a post-hoc student t-test were used for analysis of data. Probability level $P < 0.05$ was regarded as significant. Statistical analysis was done with the aid of computer software SPSS 2007 and Microsoft Excel 2007 for Windows vista (Brain Series, China).

3. RESULTS

3.1 Phytochemistry

Table 1 shows the result of phytochemical analysis of ethanol extract of *G. kola*(bitter kola) seeds. The extract contained alkaloids, saponins, tannins, flavonoids, cyanogenic glycosides, sterols and phenols in moderate to high quantities. Caffeine was not present.

Table 1. Phytochemical analysis of *Garcinia kola*

Phytochemical Compounds	Test	Observation	Inference	%
Alkaloids	Wagner's Dragendorff's reagent Test	Reddish-brown precipitate	+++	1.32
Saponins	Frothing Test	Stable Frosh emulsion	++	3.35
Tannins	Acid Test	Reddish-brown colour	++	0.85
Flavonoids	Sodium hydroxide Test	Yellow colour	++	0.48
Cyanogenic glycosides	Sodium picrate Test	Red colour to brown colour	+++	13.80
Sterols	Salkowkis Test	Red colour at interface	++	0.19
Phenols	Ferric chloride Test	Greenish-brown precipitate	++	0.35

Key: +++ ----------------------------------Highly present,
++ -----------------------------------Moderately present

Table 2 shows the result of the chemical analysis of ethanol extract of Nescafe coffee. The analysis shows that the Nescafe coffee contained alkaloids, saponins, tannins, flavonoids, phenols and caffeine in moderate to slight quantities. While caffeine was found in moderate quantities (++ or 4.72%), the others were found in slight quantities. There were no cyanogenic glycosides and steroids present unlike in *Garcinia kola* where they were present in moderate quantities.

Table 2. Phytochemical analysis of Nescafe coffee

Phytochemical Compounds	Test	Observation	Inference	%
Alkaloids	Wagner's Dragendorff's Test	Reddish-brown precipitate	+	0.86
Saponins	Frothing Test	Stable Frosh emulsion	+	0.45
Tannins	Acid Test	Reddish-brown colour	+	0.59
Flavonoids	Sodium hydroxide Test	Yellow colour	+	0.19
Cyanogenic glycosides	Sodium picrate Test	Red colour to brown colour	-	-
Sterols	Salkowkis Test	Red colour at interface	-	-
Phenols	Ferric chloride Test	Greenish-brown precipitate	+	0.03
Caffeine	TLC, HPLC		++	4.72

Key:++ -- Moderately present,
+ -------------------------------------- Slightly present

3.2 Lethality Study for *Garcinia kola* and Coffee (Nescafe)

The graded percentages of *Garcinia kola* diet (10% to 70% *G. kola* diet) fed to seven groups of mice (n=6 per group)did not show any lethality. So, the 30% *Garcinia kola* diet (30g of *G. kola* mixed with every 70g of rodent chow) given to the test animals was considered safe for the experiments. However, the ethanol extract of *G. kola* administered orally in another set of mice, showed lethality at high doses and probits were obtained and plotted against log dose concentration. The LD_{50} for orally administered *G. kola* was calculated from the chart as 682.18mg/kg body weight (Fig. 1).

Graded percentages of coffee diet (0.5% to 10% of coffee diet) were fed to 7 groups of mice (n=6 mice per group) but no deaths were recorded. So, the 2% coffee diet (2g of coffee mixed with every 98g of rodent chow) given to the test animals was considered safe for the animals. However, when various doses of ethanol extract of coffee were administered orally to another set of animals, the LD_{50} established as 3121.76mg/kg body weight (Fig. 2).
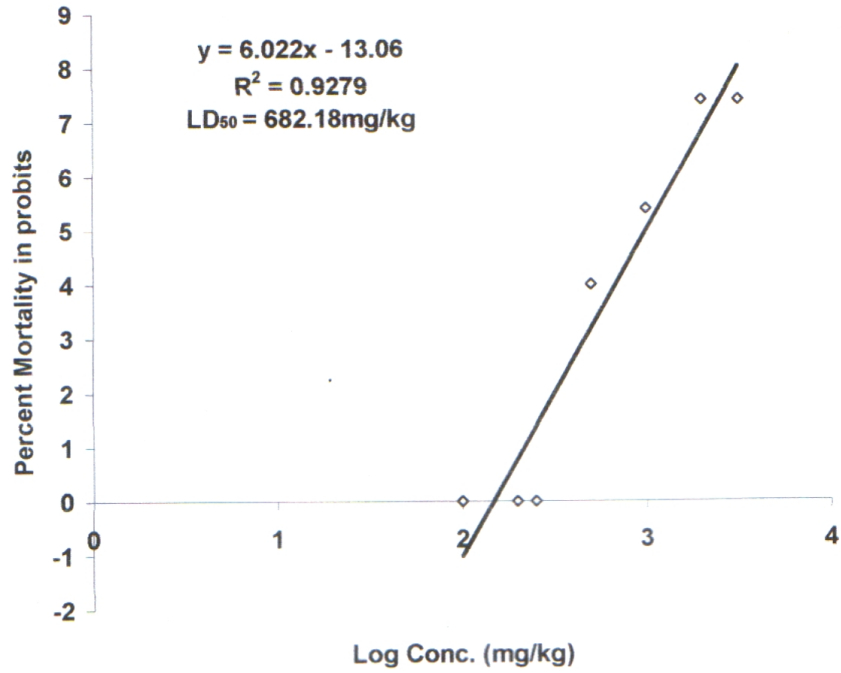
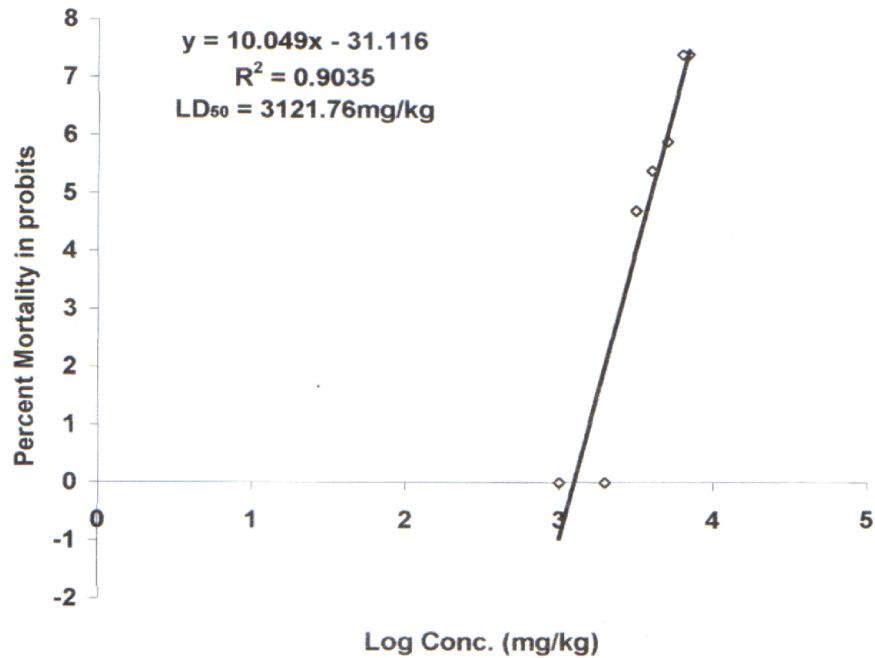**Fig. 1. Lethality (LD$_{50}$) study of ethanol extract of *Garcinia kola* seeds in mice**

Fig. 2. Lethality (LD$_{50}$) study of ethanol extract of Nescafe coffee in mice
Comparison of mean food intake between mice fed G. kola, coffee and control diets

3.3 Comparison of Mean Food and Water Intake between Mice fed *G. kola*, Coffee and Control Diets

Fig. 3 shows that the mean food intake of the control, *G. kola* diet fed and coffee diet-fed mice were 9.67 ± 0.14g, 9.34 ± 0.15gand 6.49 ± 0.11g respectively. The mean food intake for the coffee group of mice was lower(P<0.001) when Compared to both the control, and the *G. kola* diet fed group of mice.
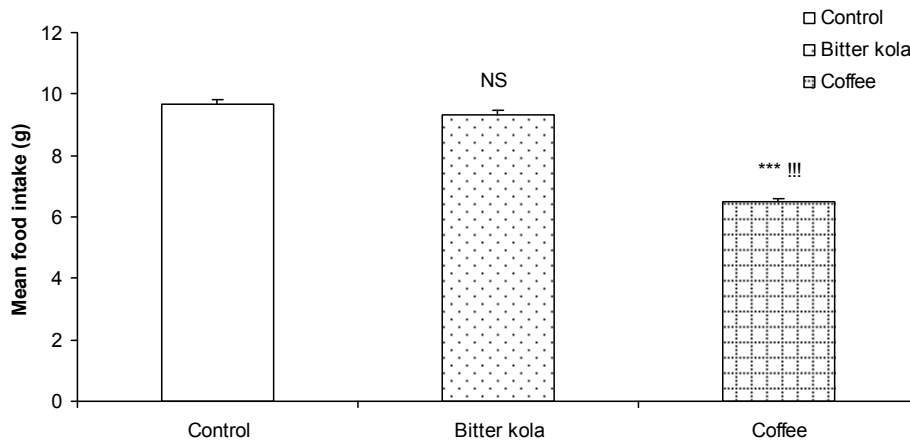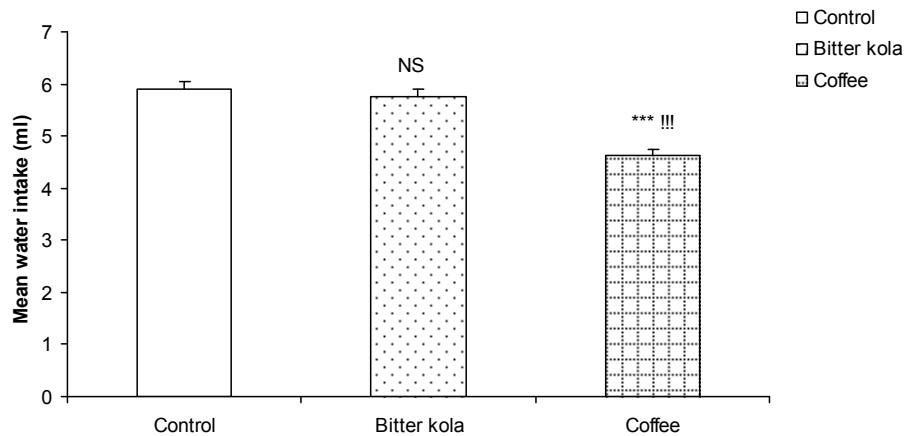

Fig. 3. Comparison of mean food intake between mice fed bitter kola, coffee and control diets respectively
NS – Not significant compared to control
*** - Significant at p< 0.001 compared to control
!!! - Significant at p< 0.001 compared to Bitter kola

The mean water intake followed a similar trend as the food intake (Fig. 4). The mean water intake for the coffee diet fed mice (4.63 ± 0.11 ml) was lower than that of the control mice (5.89 ± 0.14 ml) and the *G. kola* diet-fed mice (5.78 ± 0.12 ml)(p< 0.001).

Fig. 4. Comparison of mean water intake between mice fed bitter kola, coffee and control diets respectively
NS – Not significant compared to control
*** - Significant at p< 0.001 compared to control
!!! - Significant at p< 0.001 compared to Bitter kola

3.4 Comparison of Body Weight Changes between Mice fed *G. kola*, Coffee and Control Diets

Fig. 5a and b compares the daily body weight and mean body weight changes in the experimental groups. The mean body weight changes of the coffee diet fed group decreased progressively after an initial increase while that of the control and *G. kola* increased progressively as shown in Fig. 5a. The mean body weight changes of the coffee diet fed group of mice (1.24 $\pm$ 1.57g) was significantly lower (P<0.001) when compared to control (9.29 $\pm$ 0.79g) and *G. kola* group (10.88 $\pm$ 1.14g). However, the body weight change did not differ between the control and *G. kola* fed groups (Fig. 5b).
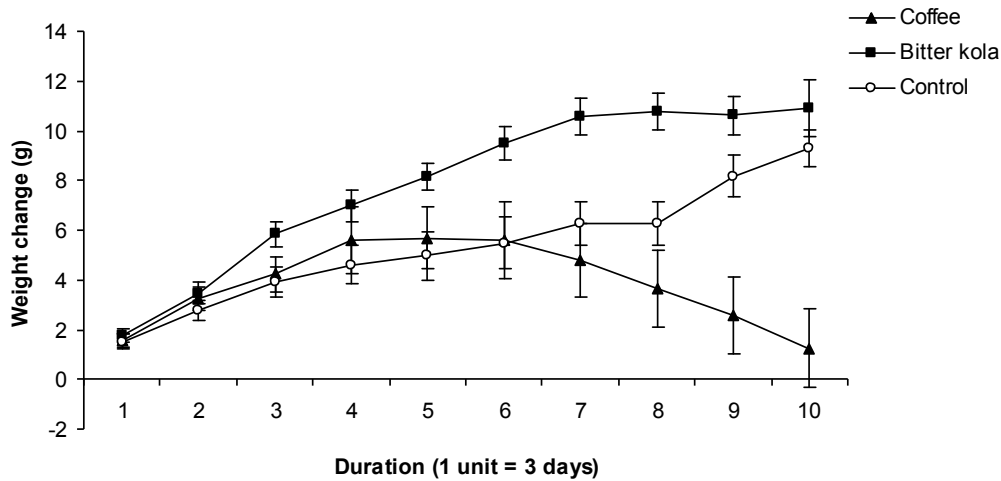

Fig. 5a. Comparison of daily body weight changes between mice fed bitter kola, coffee and control diets
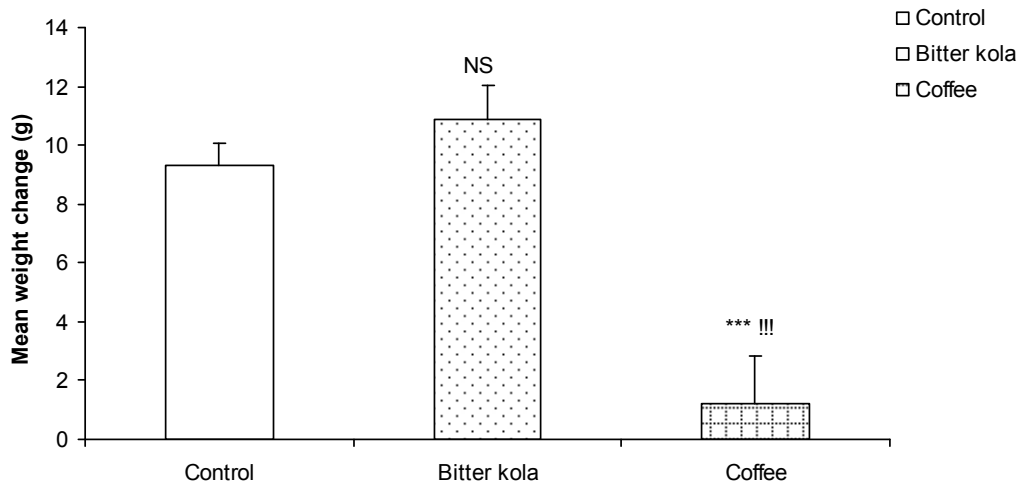

Fig. 5b. Comparison of mean body weight changes between mice fed bitter kola, coffee and control diets

NS – Not significant compared to control
*** - Significant at p< 0.001 compared to control
!!! - Significant at p< 0.001 compared to Bitter kola

3.5 Swim Latencies during Acquisition and Reversal Training in the Morris Water Maze

The swim latencies of the bitter kola diet fed mice for the three days of acquisition training were not significantly different compared to control. However, the swim latencies of the coffee diet fed mice was significantly shorter (P<0.01) compared to both kola and control diet fed groups of mice (Fig. 6). During reversal training, the swim latencies of the coffee group was similarly lower (P<0.01) compared to both the control and the bitter kola fed mice (Fig. 7).
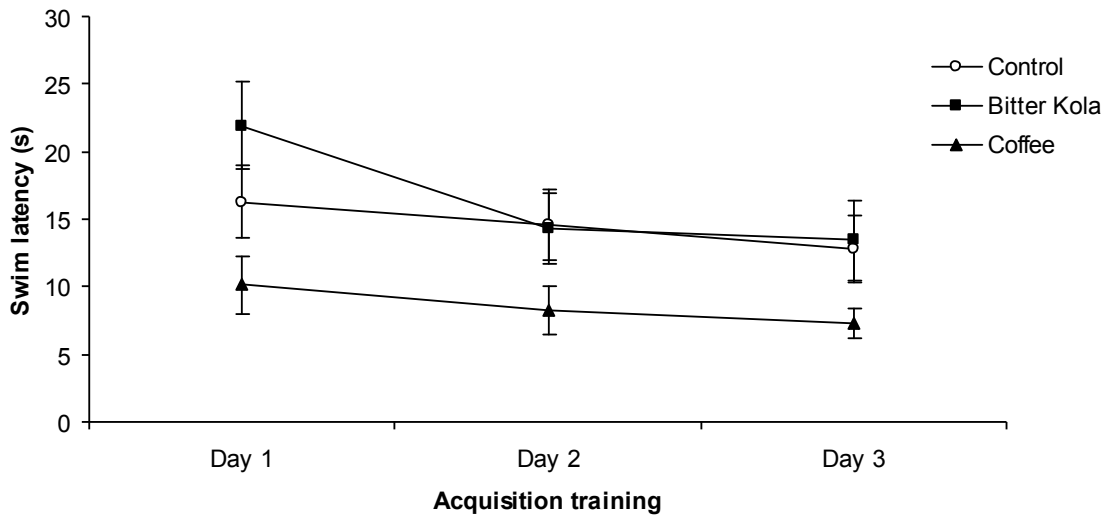

Fig. 6. Comparison of swim latencies during the acquisition training in the Morris water maze between mice fed bitter kola, coffee and control diets respectively
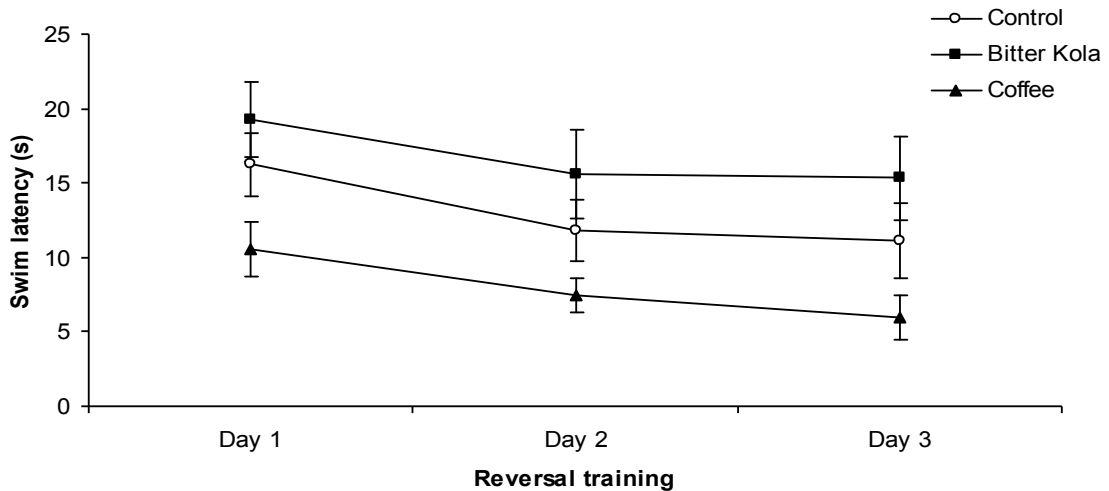

Fig. 7. Comparison of swim latencies during the reversal training in the Morris water maze between mice fed bitter kola, coffee and control diets respectively

3.6 Quadrant Duration during the Probe Trial in the Morris Water Maze

Fig. 8 compares the quadrant duration during the probe trial in the Morris water maze between the 3 experimental groups of mice. During the probe trial, the NW quadrant duration (time in the quadrant with hidden platform during acquisition training) did not differ significantly between the groups, even though it tended to be higher in the coffee group. However, the time spent swimming in the SE quadrant (the quadrant with hidded platform during the reversal training – also called retention quadrant) was significantly more for the coffee diet-fed group of mice when compared to both control and *G. kola* diet-fed mice (P<0.05).
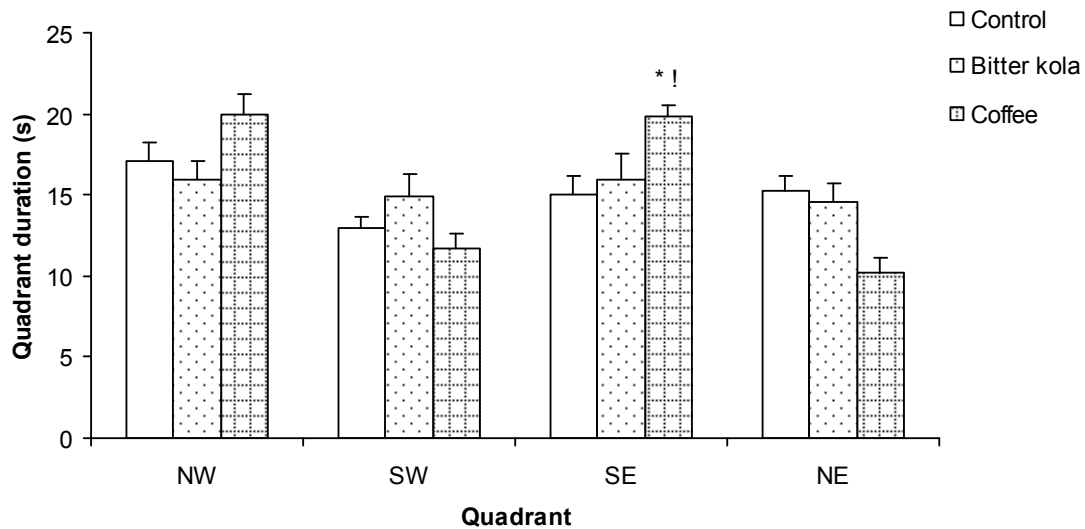

Fig. 8. Comparison of quadrant during the probe trial in the Morris water maze between mice fed bitter kola, coffee and control diets respectively
* - Significant at p< 0.05 compared to control;
! - Significant at p< 0.05 compared to Bitter kola

3.7 Swim Latencies during the Visible Platform Task in the Morris Water Maze

Fig. 9 compares the swim latencies between the mice fed bitter kola, coffee and control diets respectively. During the visible platform task, the swim latency of the bitter kola diet-fed mice was not significantly different compared to control. However, the swim latency of coffee fed mice was significantly lower compared to control diet group (p< 0.05) and bitter kola diet group(p<0.01).
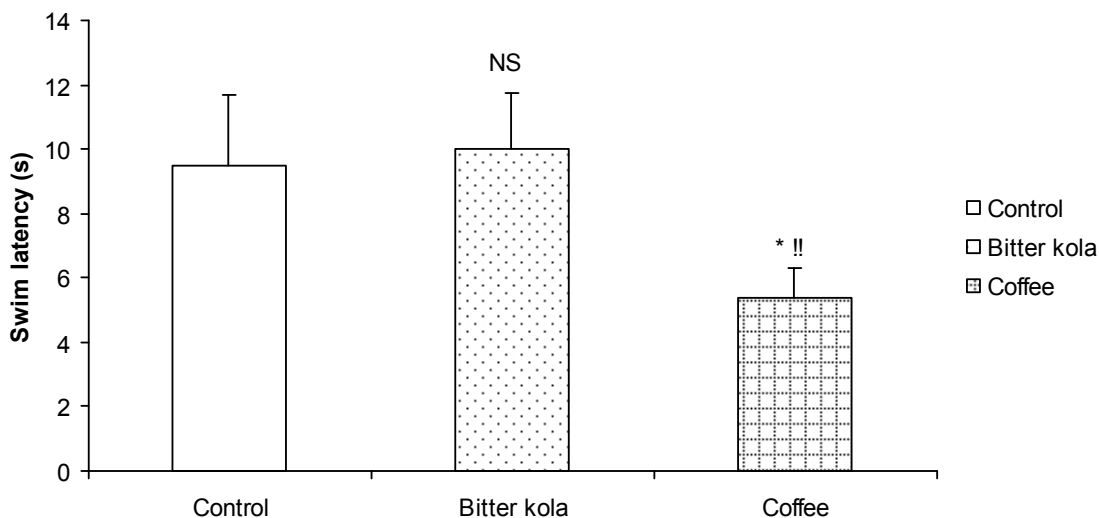
Fig. 9. Comparison of swim latencies during the visible platform task in the Morris water maze between mice fed bitter kola, coffee and control diets respectively
NS – Not significant compared to control
* - Significant at p< 0.05 compared to control
!! - Significant at p< 0.01 compared to Bitter kola

4. DISCUSSION

4.1 Chemical Analysis of *Garcinia kola* and Coffee (Nescafe)

The phytochemical screening of *Garcinia kola* showed that it contains high quantities of alkaloids, and moderate quantities of saponins, tannins, flavonoids, cyanogenic glycosides, sterols and phenols. No traces of caffeine were found in the *Garcinia kola* seeds. This result obtained is against the existing but unproven belief that *Garcinia kola* contains caffeine. On the other hand, the phytochemical analysis of coffee (Nescafe) revealed a high percentage of caffeine (4.72%). Other chemicals substances present in slight to moderate quantities were: alkaloids, saponins, tannins, flavonoids, cyanogenic glycosides, sterol and phenols.

4.2 LD_{50} of *Garcinia kola* and Coffee

From the study, the LD_{50} for orally administered ethanol extract of *G. kola* was 682.2mg/kg body weight. The LD_{50} for orally administered coffee was 3121.8mg/kg body weight. When graded percentages of *Garcinia kola* diet (10% to 70%) and coffee diet (0.5% to 10%)were given, no lethality was recorded. Thus the 30% *G. kola* diet given to the *Garcinia kola* group of mice was safe for the experiment. Similarly, the 2% coffee diet given to the coffee diet group of mice was considered safe for the animals.

4.3 Food and Water Intake

The mean food intake of the *Garcinia kola* diet-fed group of mice did not differ significantly compared to control. However, the mean food intake of the coffee diet-fed group of mice was significantly lower compared to *G. kola* and control diets fed group of mice. Food intake is

controlled by feeding centers in the hypothalamus. An animal looks for food to eat when the hunger center (in the lateral hypothalamus) is stimulated, and stops eating when the satiety center in the ventral medial hypothalamus is stimulated and at same time the hunger center is been inhibited [21]. It is possible that the coffee inhibited the hunger center resulting in reduced food intake in mice. These results agree with those of Bisonget al. [22] who also showed that caffeine in kola nuts reduced food intake in mice.

The mean water intake followed a similar trend as the food intake, with water intake being lower in the coffee diet fed group of mice compared to *G. kola* diet-fed and control groups. Water intake in the *G. kola* diet fed group of did not differ from control. Hyperosmolarity in blood stimulates hypothalamic osmoreceptors thus increasing thirst and drive to drink water [23]. Inhibition of these hypothalamic thirst centers will decrease thirst and therefore drinking. It is also possible that the caffeine in the coffee diet inhibited the hypothalamic thirst center causing a decrease in water intake.

4.4 Body Weight Change

The body weight change during the experiments did not differ between the *Garcinia kola* diet fed mice and control. However, the mean body weight change of the coffee diet-fed group of mice was lower than control and the *G. kola* diet-fed mice. Although not significantly so, this result tows the line of earlier studies by Adedeji et al. [24] who reported a slight weight gain after feeding pullet chicks with dry seed powder of *G. kola*. On the other hand, other researchers have also shown that coffee intake decreased body weight by decreasing food intake via some indirect mechanism that is not well understood [11].

4.5 Learning and Memory

The brain is unique for its ability to add to its stock of information by acquiring information, (learning), retaining and retrieving the information (memory) as appropriate [25,21].The hidden platform version of the Morris water maze tests forvisuo-spatial learning and memory, which is hippocampus dependent [26]. Thus, stimulation or inhibition of hippocampal neurons would affect learning and memory. The visible platform version of the Morris water maze, is a non-hippocampal task, which is dependent on the caudate nucleus and putamen of the basal ganglia. The visible platform uses a unique intra-maze visual cue that is placed at the location of the escape platform whereas the visuo-spatial learning task uses extra-maze cues [26].

Mice which learn faster would locate the hidden platform earlier than their counterparts i.e. shorter swim latencies. The results in this study showed that during the 3-day acquisition and 3-day reversal training, the swim latencies were shorter in the coffee diet-fed mice compared to *G. kola* and control diet-fed groups, which did not differ between each other. This observation suggests better learning in the coffee group. The swim latencies during the visible platform task did not show any difference between the *G. kola* diet-fed and control groups, which indicates that there were no visual impairments in any of these mice. The swim latency for the coffee diet-fed mice was however lower compared to both control and the *G. kola* diet-fed mice. This is however not due to any visual impairment but ability of caffeine to suppress locomotor activity when taken in large doses.

During the probe trial of the Morris water maze test, the reversal quadrant is the retention quadrant. So, it is expected that mice which have learned the position of the hidden platform

during the reversal training would spend more time exploring the reversal (retention) quadrant in search of the hidden platform. The mice in the three groups showed some preference for both the North-West (NW) quadrant (platform location during acquisition training) and the South-East quadrant (platform during location during reversal training), which showed that there has been retention of the tasks (memory). However, the NW quadrant duration did not differ between the three groups of mice. The South-East quadrant duration was significantly higher for the coffee diet-fed group compared to both control and bitter kola group. Thus, the coffee diet-fed mice had better visuo-spatial memory.

The overall performance in the Morris water maze showed that the coffee group of mice had better performance during the acquisition and reversal training which showed a better learning ability than the *G. kola* diet-fed mice and control. The performance of the *G. kola* diet fed mice did not differ from control. Also, the result obtained from the probe/retention trial showed that the coffee fed group of mice had a better memory than the *G. kola* dietfed group of mice and control. The enhancement of learning and memory in the coffee diet-fed group of mice may be attributed to one of its major constituents, caffeine, which is a stimulant of the central nervous system that has been shown to improve learning and memory [27]. It is possible that caffeine facilitates the consolidation of memory because it blocks one of the receptors for adenosine, a neurotransmitter that seems among other action to dull concentration [27].

These results on the positive effect of coffee diet on learning and memory may not be attributed to the observed decrease in food and water intake, and body weight change as these have not been shown in earlier studies to affect learning and memory. These also did not negatively affect learning and memory in this study.

5. CONCLUSION

In conclusion, the *G. kola* diet did not affect both learning and memory. This is against the belief that it has a stimulant effect on the nervous system and also against the belief that it affects concentration and therefore, learning and memory. It is possible that these effects are borne out of some psychological effect resulting from the socio-cultural importance placed on the *G. kola* seeds.

CONSENT

Not applicable.

ETHICAL APPROVAL

The authors herein declare that the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) as well as national laws on the care of animals were strictly adhered to during the experiments. Appropriate approval was also obtained from the local ethical committees.

COMPETING INTEREST

The authors hereby declare that there was no conflict of interest.

REFERENCES

1. Onochie R, Stanfield A. Properties of *Garcinia kola* seed: A general belief that *Garcinia kola* contains caffeine. J Environ Neurosci Biomed; 1960.
2. Atawodi S, Mende P, Pfundstein B. Nitrosaableamarines and nitrosamide formation in natural stimulants, *cola acumimata, c. nitida* and *Garcinia kola* food chain. Toxical. 1995;33(8):625-30.
3. Plowden CC. A manual of plants names. 3rd ed. London, George Ltd.1972; pp. 239.
4. Braide VB, Vittrotio G. Histological Alterations by a diet containing seed of *Garcinia kola*: Effect on liver, kidney, and intestine in rat. Gedenbauers Morhol. Jahrbuch Leipzig. 1989;1334. I S.95-101
5. Orie NN, Ekon EU. The Broncho-dilatory effect of *Garcinia kola* seeds. East African Med. J. 1993;70(30):143-145.
6. Iwu MM, Igboko O. Flavoniods of *Garcinia kola* seeds. J. Natural Prod. 1982;45:650-51.
7. Iwu MM, Igboko OA, Okunji CO, Tempesta MS. Antidiabetic and aldose reductase activities of Biflavonones of *G. kola.* J. Pharm Pharmacol. 1990;42:290-292
8. Hussain RA, Owegby AG, Waterman P*G. kola* mone, a novel polyisoprenylated benzophenone with antimicrobial properties from the fruit of *Garcinia kola*. Pak J Med Sci. 1982;44:78-81
9. Braide VB. Anti-hepatotoxic biochemical effects of Kolaviron, a bioflavonoid of *Garcrinia kola.* Phytotherapy. Res. 1991;5(38):35-37.
10. Okunji CO, Iwu MM. Molluscidal activity of *G. kola* biflavanones Floterapia. 1991;62:74-76
11. Fisone GG, Borgkurt A, Usiello A. Caffeine as a psychoactive stimulant; mechanism of action; Cell Mol. Life sc. 2004;61(7-8):857-72.
12. Nehlig A. Are we dependent upon coffee and caffeine? A review on human and animal data. Neuro. Sci. Biobehav. Rev. 1999;23(4):563-576.
13. Farinu GO. *G. kola* and the Brian: Journal of Animal and Veterinary Advances. 2006;5(3):18 –187.
14. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol.1983;54:275-28.
15. Kumar D, Bhat ZA, Shah MY. Anti-anxiety activity of successive extracts of Angelica archangelica Linn. on the elevated T-maze and forced swimming tests in rats. J Tradit Chin Med. 2012;32(3):423-9.
16. Harborne JB. Isolation and extraction of phytochemicals, 4[th]edu, chapman and Had, 733rd Avenue, New York. 1998;1017.
17. Obadoni BO, Ochuko I. Phytchemical status and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure Appl. Sci. 2001;8:203 – 208
18. Van-Burden TP, Robinson WC. Formation of complexes between protein and tannic acid. J. Agric. Food Chem. 1981;1:72–82.
19. Mohammed MJ, Al-Bayati FA. Isolation, identification and purification of caffeine from *Coffea arabica* L. and *Camellia sinensis* L.: A combination antibacterial study. IJGP (International Journal of Green Pharmacy). 2009;3(1):52-57.
20. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience methods. 1984;11:47-60
21. Ganong WF. Review of Medical Physiology. McGraw Hill Baston 21[st]ed.GeorgeLtp. 2005;pp 239.
22. Bisong SA, Joshua IE, Okon VE, Osim EE. Effect of chronic consumption of kola nut on learning and memory in mice. Nig. J. Physiol. Sci. 2008;23(1-2):132.

23. Fox SI. Human Physiology.9th edition. Pierce College, New York, Woodland hills; 2006.
24. Adedeji OS, Farinu GO, Ameen SA, Olayeni TB. The Effects of Dietary bitter kola (*Garcinia kola)* inclusion on Body Weight, Heamatology and Survival Rate of Pullet Chicks. Journal of Animal and Veterinary Advances. 2006;5(3):184-187.
25. Osim EE. Neurophysiology 2nd ed. Calabar, El Sapphire Limited. 2003;pp.198
26. McDonald RJ, White NM. Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. Behavioural Neural Biology.1994;61:260–270.
27. Holloway M. Seeking smart drugs. Scientific American. 1998;9(40):39-43.