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Full Length Research Paper

The comparative effects of chronic consumption of kola nut (*cola nitida*) and caffeine diets on exploration, anxiety and fear in Swiss white mice

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The present study was designed to investigate the comparative effects of chronic consumption of kola nut (*Cola nitida*) and caffeine-diets on exploration, anxiety and fear related behaviours in Swiss white mice. The study was carried out on 30 adult Swiss white mice of both sexes weighing 15.0 to 30.0g using the open field apparatus, the light/dark transition box and the elevated plus-maze. The frequency of rearing for mice fed kola nut diet did not differ significantly compared to control, this was significantly lower (P<0.01) in the caffeine-fed mice compared to control. This trend was also similar for grooming. The kola nut and the caffeine-fed mice spent more time in the light portion of the light/dark transition box than in the dark portion of the box when compared to the control (P<0.01). The frequency of stretch-attend postures in both the light and dark portions of the light/dark transition box were significantly lower for both the kola nut-fed and caffeine-fed mice when compared to the control group (P<0.01). In the elevated plus-maze, the duration of entry into the open arm for the kola nut-fed (P<0.05) and caffeine-fed (P<0.01) were significantly higher when compared to the control. This was lower in the close arm for the kola nut-fed (P<0.05) and caffeine-fed (P<0.01) when compared to the control. These results indicate that chronic consumption of kola nut and caffeine decrease anxiety and fear related behaviours in Swiss white mice. However, caffeine diets-fed mice seemed to have a more potent effect in decreasing anxiety and fear while increasing exploration. Hence, the effect of kola nut on exploration, anxiety and fear may not be due to caffeine only.

Key words: Kola nut, caffeine, exploration, anxiety and fear.

INTRODUCTION

Kola nut is one of the most common mastigatory in Nigeria (Umoren *et al*, 2009). It is commercially grown in the West where it is known as *Obi* in Yoruba, consumed by the Northerners where it is known as *Goro* in Hausa, and revered in the East where it is called *Oji* in Igbo. It is of great importance in the traditional institution hence the adage by the Igbos "He who brings Kola brings life". Kola is generally ascribed to elder's privileges. The nuts are either chewed whole or used in powdered state. It is known to cause mild stimulation of the central nervous system and produce a temporal feeling of increased physical strength often associated with a reduction of hunger and fatigue, which may be due to its high content of carbohydrates (Martin *et al*., 1983).

Preliminary studies on the use of kola nuts for soft drink production by (Jayeola, 2001) in the Cocoa Research Institute of Nigeria indicated that fresh kola nut contains 8.90% Protein, 0.92% Fat, 2.4% Ash and 1.50% Caffeine. (The Psychoactive Encyclopedia, 2011) also reports the key chemical constituents of kola nut to include: caffeine, theobromine, tannins and phenolics, including d-catechin, 1-epicatechin, and kolanin. It also contains phlobaphens, proteins and starch. Interest in the physiologically active agent led to the identification of

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alkaloids in kola nut. It is considered that there are unstable complexes which occur in fresh kola nuts and these are the kolanins, tannins and caffeine glycosides. These complexes oxidize and hydrolyze to form kola red and free caffeine under the influence of enzymes when the nuts are drying out. If these enzymes are inactivated prior to drying the seeds, for instance with heat treatment, then this process will not occur and the dried seeds will retain their physiological actions. Investigations show that, caffeine is partly free and partly in the above mentioned complexes. The methylxanthines all share the ability to relax smooth muscle, stimulate the CNS, and produce diuresis. Theophylline is the most potent of the methylxanthines for relaxing bronchial muscles, taking it effective as an asthma treatment. Caffeine is also a bronchodilator that increases minute ventilation in patients with chronic obstructive lung disease and has been utilized as a respiratory stimulant in neonates with recurrent apneic episodes (Murat *et al*., 1981 and Gong *et al*., 1986). Many coffee drinkers report acid reflux symptoms. Caffeine stimulates gastric secretion of acid and pepsin, probably by antagonizing the inhibitory actions of adenosine on acid secretion by parietal cell (Gerber *et al*., 1985). Caffeine may also decrease lower esophageal sphincter pressure.

Adenosine plays a physiological role in inhibiting lipolysis, which could be undermined by its receptor antagonism. Caffeine increases free fatty acids, cortisol, and blood glucose. Lipid metabolism may be affected by caffeine indirectly through an effect of increased circulating catecholamines stimulating lipolysis and release of free fatty acids (Williams *et al*., 1985). Caffeine is known to be a fat burner and beneficial in assisting weight loss (Blades, 2000). It is generally believed that most of the actions of kola nuts are attributable to caffeine. Inspite of many actions of kola nuts and caffeine, very limited work has been reported on their effects on exploration, anxiety and fear. Since they are nervous system stimulants, it is likely that they may affect exploration, anxiety and fear. Therefore, this study determines and compares the effect of chronic consumption of kola nuts and its active principle, caffeine on exploration, anxiety using Swiss white mice.

MATERIALS AND METHODS

Preparation of kola nut

Fresh kola nuts (*Cola nitida*) were obtained from the Bogobiri market, Calabar, Nigeria and used for the study. The kola nuts were washed, and dried at 60^0c for 12 hours, and ground using electric Kenwood blender. Twenty-five grams (25g) of the kola nut was mixed with 75g of normal rat chow feed in a ratio of 1:3 by weight respectively (Osim and Udia, 1998). A mortar/pestle was used to blend the mixture to form the kola nut-diet. The kola nut-diet was administered to group B on daily basis for 28 days.

Preparation of caffeine

Synthetic caffeine, a white powder crystalline substance was obtained from May and Baker (M&B) United Kingdom was used for the study. Kola nuts were dried to constant weight in an oven at 60^oc. From the estimation of the caffeine content of kola nuts according to the method used by (Somorin, 1973). and confirmed by (Eka, 1971). 60g of the dry nuts used for these studies contained about 1.0g of caffeine. A caffeine-diet was prepared by using a spatula and a weighing balance to measure 1.0g of the powdered caffeine, mixed with 150g of normal rat feed. So, the amount of caffeine added was equivalent to that contained in the kola nuts given previously. A mortar/pestle was used to blend the mixture to form the caffeine-diet. This preparation was done each week (for a period of 4 weeks). The caffeine-diet was administered to group C on daily basis.

Experimental Animals

Thirty (30) adult Swiss white mice weighing between 15-30g obtained from the disease free stock of the animal house, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria were used for the study. The animals were randomly assigned into three (3) groups of ten (10) animals per group. Each mouse in a study group was individually housed in a plastic cage with iron gauze bottom grid and a wire screen top. The animal room was adequately ventilated, and kept at room temperature and relative humidity of 26 ± 2^0c and 40-70% respectively with 12 hour natural light-dark cycle.

Treatment Regimen

The animals were fed *ad libitum* with water and normal rat chow (Livestock feeds Nig. Ltd., Lagos, Nigeria). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. The control group (A) received normal rat feed and clean drinking water freely. The kola nut-diet group (B) received normal rat feed mixed with ground dried kola nut as described below and allowed clean drinking water freely. The caffeine-diet group (C) received normal rodent feed mixed with powdered caffeine as described below and allowed clean drinking water freely. The experiments were conducted between the hours of 1.00pm and 2.00pm daily, and the chronic studies (28 days) were

carried.

The open-field apparatus

The open field test provides simultaneous measures of locomotion, exploration and anxiety (Walsh and Cummins, 1976). Behaviours such as the number of line crosses and the frequency of rearing are used as measures of locomotor activity, but are also measures of exploration and anxiety. A high frequency of these behaviours indicates increased locomotion and exploration and/or a lower level of anxiety.

The light/dark transition (LD) box

The light and dark transition box is a test of unconditioned anxiety and exploratory behaviour. It is based on the conflict between exploring in a novel environment and avoidance of bright light (Bourin and Hascoet, 2003). Exploration activity in the light area can be increased through the use of anxiolytic drugs (Coastall *et al.,* 1989). Increased activity (line crosses, rearing) and transactions between the light and dark chambers is associated with non-anxious behaviour (Kim *et al*., 2002; Bourin and Hascoet, 2003; Raud *et al.,* 2005). Each mouse was picked up using a plastic bucket and placed in the centre division of the large compartment facing the door. The mouse was allowed to explore the transition box for 5 minutes. Entering into a chamber is defined as the placement of all four paws in the chamber. Behaviours scored included the following: transitions, chamber duration and stretch-attend postures.

The elevated plus-maze (EPM)

The EPM is a test of anxiety and exploration in rats and mice. Each mouse was placed in the centre square of the EPM facing an open arm and its behaviour recorded by an observer using a stop watch. The behaviours scored include: open arm duration and closed arm duration.

Statistical Analysis

Data collected were expressed as mean ± standard error of mean (SEM), analysis of variance (ANOVA) and the student "t" test were used for analysis. Values of $p<0.05$ were regarded as significant.

RESULTS

Open field apparatus

The frequency of rearing for mice fed kola nut diet was 44.50 ± 4.44/5min session. This was not significantly different when compared to control 44.00 ± 4.87/5min session. However, the frequency of rearing for mice fed caffeine diet 24.44 ± 3.27/5min was significantly lower ($P<0.01$) compared to control. This was also significantly lower compared to the frequency of rearing for mice fed kola nut ($P< 0.01$).

The frequency of grooming in the kola nut fed mice was 2.75 ± 0.47/5min session. This was not significantly different from control group which was 2.56 ± 0.38/5min session. However, the frequency of grooming for mice fed caffeine diet was 1.71 ± 0.25/5min session. This was significantly lower ($P<0.05$) compared to control. This was also significantly lower when compared to the frequency of grooming for mice fed kola nut ($P< 0.05$).

Light/dark transition box

The frequency of line cross in the light chamber of the light/dark (LD) transition box for kola nut fed mice was 56.25 ± 12.46/5min session. This was not significantly different when compared to control which was 38.60 ± 7.99/5min session. The frequency of line cross in the dark chamber of the light/dark transition box for kola nut fed mice was 50.25 ± 10.51/5min session. This was also not significantly different from the control which was 67.11 ± 6.03/5min session.

The frequency of line cross in the light chamber of the LD transition box for caffeine diet-fed mice was 66.33 ± 9.71/5min session. This was significantly higher ($P<0.05$) when compared to control which was 38.60 ± 7.99/5min session. Also, the frequency of line cross in the dark chamber of the LD transition box for mice fed caffeine diet was 40.33 ± 6.22/5min session. This was also significantly lower ($P<0.01$) when compared to control which was 67.11 ± 6.03/5min session. Moreover, this was not significantly different when compared to the frequency of line cross in the dark transition box for mice fed kola nut.

The duration in the light portion of the box for kola nut fed mice was 139.50 ± 42.28/5 min session. This was not significantly different from control which was 110.10 ± 26.92/5 min session. Also, the duration in the dark portion of the box in the kola nut fed group was 160.50 ± 42.28/5 min session. This was also not significantly different

Table showing summarized result of the different parameters used in scoring exploration, anxiety and fear related behaviours in Swiss white mice

Open field apparatus	**Control group**	**Kolanut-diet**	**Caffeine-diet**
Frequency of rearing	44.00±4.87/5mins	44.50±4.44/5mins	24.44±3.27/5mins* (P<0.01)
Frequency of grooming	2.56±0.38/5mins	2.75±0.47/5mins	1.71±0.25/5mins* (P<0.05)
L/D Trans. Box			
Frequency of line cross (light portion)	38.60±7.99/5mins	56.25±12.46/5mins	66.33±9.71/5mins* (P<0.05)
(dark portion)	67.11±6.03/5mins	50.25±10.51/5mins	40.33±6.22/5min* (P<0.01)
Chamber duration (light portion)	110.10±26.92/5mins	139.50±42.28/5mins	193.00±22.5/5mins* (P<0.01)
(dark portion)	211.00±16.08/5mins	160.50±42.28/5mins	109.40±23.96/5mins* (P<0.01)
Stretch attend posture (light portion)	11.50±3.89/5mins	3.50±1.32/5mins* (P<0.05)	7.00±1.27/5mins
(dark portion)	20.22±5.45/5mins	5.00±0.71/5mins* (P<0.01)	5.11±1.88/5mins* (P<0.01)
Elevated plus maze (open arm)	42.78/5min	147.75/5mins* (P<0.05)	126.78/5mins* (P<0.01)
(closed arm)	257.25/5mins	152.255/5mins* (P<0.05)	173.22/5mins* (P<0.01)

Each value represents mean ± SEM, for frequency spent in the open field apparatus, light/dark transition box and elevated plus maze in 10 Swiss white mice. * (P<0.01 and P<0.05 respectively) vs control.

when compared to control which was 211.00 ± 16.08/5 min session.

The duration in the light portion of the box for caffeine fed mice was 193.00 ± 22.55/5 min session. This was significantly higher (P<0.01) when compared to control which was 110.10 ± 26.92/5 min session. However, the duration in the dark portion of the box for caffeine fed group was 109.40 ± 23.96/5 min session. This was also significantly lower (P<0.01) when compared with control 211.00 ± 16.08/5 min session. Moreover, there was no significant difference in the chamber duration in both the Light and dark transition box for the groups of mice fed kola nut and caffeine diet.

The frequency of stretch-attend postures in the light portion of the box for kola nut fed mice was 3.50 ± 1.32/5min session. This was significantly lower (P<0.05) when compared to control which was 11.50 ± 3.89/5 min session. Also, the frequency of stretch-attend postures in the dark portion of the box for kola nut fed mice was 5.00 ± 0.71/5min session. This was significantly lower (P<0.01) when compared to control which was 20.22 ± 5.45/5min session.

The frequency of stretch-attend postures in the light portion of the box for caffeine fed mice was 7.00 ± 1.27/5min session. This was not significantly lower when compared to control which was 11.5 ± 3.89/5min session. However, the frequency of stretch-attend postures in the dark portion of the box for caffeine fed mice was 5.11 ± 1.88/5min session. This was significantly lower (P<0.01) when compared to control which was 20.22 ± 5.45/5min session. Moreover, the duration of stretch-attend postures in both the light/dark portions of the box for caffeine-fed mice was not significantly different when compared to the duration of stretch-attend postures in the light/dark portions of the box for kola nut fed mice.

Elevated plus maze

The duration of entry into the open arm of the elevated plus maze for kola nut fed mice was 147.75/5min session. This was significantly higher (P<0.05) when compared to control which was 42.78/5min session. The duration of entry into the closed arm of the elevated plus maze for kola nut fed mice was 152.25/5min session. This was significantly lower (P<0.05) when compared to control 257.22/5min session.

The duration of entry into the open arm of the elevated plus maze for caffeine fed mice was 126.78/5min session. This was significantly higher (P<0.01) when compared to control 42.78/5min session. The duration of entry into the closed arm of the elevated plus maze for caffeine fed mice was 173.22/5min session. This was significantly lower (P<0.01) when compared to control 257.22/5min session. Moreover, there was no significant difference in the duration of entry into the elevated plus maze between kola nut and caffeine diet-fed mice. (Table)

DISCUSSION

In order to assess the comparative effects of chronic (28 days) consumption of kola nut (*Cola nitida*) and caffeine diets on exploration, anxiety and fear related behaviour in Swiss white mice, the open field apparatus, the light and dark transition box (LD) and the elevated plus maze (EPM) were employed. The open field apparatus is in line with (Brown *et al*., 1999; Archer, 1973; Rogers, 1997; Streng, 1974). who used the open apparatus to assess the behaviour of animals in a novel environment, as well as locomotion and exploration. The behaviours scored in this study included rearing in the open, and grooming. The light and dark (L/D) transition box was used to assess the innate aversion of rodents for brightly illuminated areas and the spontaneous exploratory behaviour of rodents in response to mild stressors that is, novel environment and light (Crawley *et al*. 1997). The anxiety and fear related behaviour scored in this study included the frequency of line cross, chamber duration and stretch- attend postures in the light and dark transition box. The elevated plus-maze (EPM) has been used and has been proven as a model for assessing anxiety and fear (Brown *et al*., 1999; Lister, 1987). This test is based on the natural aversion of rodents for space and heights. Therefore, when exposed to the elevated plus-maze, fearful mice (animals) will avoid the open arm and spend most time in the closed arm (Trullas *et al*., 1993). These methods were used to assess the effect of chronic consumption of kola nut and caffeine diets on exploration anxiety and fear related behaviours in Swiss white mice.

The increased exploration and decreased anxiety and fear related behaviours in Swiss white mice following chronic consumption of kola nut and caffeine is in consonance with the reports of (Umoren *et al.,* 2009; Johnson *et al.,* 1990; Victor *et al.* 1981). which showed that the excitatory behaviour which may lead to increased exploration of the animals is due to the possible stimulatory effects of the central nervous system. The results is also in consonance with the reports of (Alan, 2009), which showed that since caffeine penetrates the blood-brain barriers, it is assumed that the central stimulant effects that enhance alertness and counteract feelings of fatigue, are due to the action of caffeine. Also, the work of (Moughan, 2002) who reported that caffeine makes people more alert, less drowsy, and improves coordination. That it is sometimes included in athlete's diets to improve physical performance. In support, the works of (Bolton *et al,.* 1981), whose research shows that caffeine use (whether in tablet form or not) results in decreased fatigue and increased attentiveness. In a similar study, (Bolton *et al.,* 1981) showed that caffeine stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses. The results of the study following chronic consumption of kola nut diet and caffeine diet is also in consonance with the reports of (Graham *et al.,* 1991) who reported that trained runners showed a 44% increase in "race-pace" endurance, as well as 51% increase in cycling endurance, after a dosage of 9 milligrams of caffeine per kilogram of body weight.

Decreased anxiety as shown by caffeine-fed mice is in line with the work of Neil (1978), which showed that excessive consumption of caffeine caused mixed depressive state in psychiatrics. The work of (Greden, 1978) also reported depressive syndrome as associated with caffeine. The reduced grooming and rearing frequency following caffeine diet consumption may also explain the withdrawal syndromes reported in animals fed caffeine diets. For instance, (Griffiths *et al,.* 1988) showed a substantial data based document in the developments of tolerance and the presence of withdrawal symptoms from chronic caffeine use.

On the other hand, the reports of (Mrvos *et al*., 1989) shows that large amounts of caffeine intake can induce anxiety severe enough to necessitate clinical attention. This however does not agree with the results of this study since chronic consumption of kola nut diet and caffeine diets caused a decrease in anxiety and fear related behaviours in mice. It could also mean that the large intake of caffeine-induced anxiety may be specie specific. Also, the reports of some investigations which claim that caffeine is anxiogenic that is, stimulate anxiety (Beach *et al.,* 1986; File and Hyde, 1979; Charney *et al.,* 1984).

Anxiety and fear are emotions controlled by the limbic system and hypothalamus (Guyton, 2006). It is likely therefore, that kola nut and caffeine affected nuclei in the hypothalamus and limbic system notably, amygdala and some nuclei in the hypothalamus to reduce anxiety and fear related behaviour in animals. The exact mechanism whereby chronic consumption of kola nuts and caffeine reduces anxiety and fear is uncertain. However, it is known that impairment of the amygdala reduces fear in animals (Guyton, 2006). It is therefore likely that kola nuts and its constituent caffeine may be impairing the amygdala.

The frequency of rearing in the open field apparatus for kola nut-fed mice did not differ significantly when compared to control. However, the frequency of rearing in the caffeine-fed group of mice was significantly lower when compared to control and kola nut-fed mice in the open field apparatus. This trend was also similar in the frequency of grooming.

The frequency of line cross in the light and dark portions of the L/D transition box for the kola nut fed mice were not significantly different from control. However, the frequency of line cross in the light portion of the L/D transition box for caffeine diet-fed mice was significantly higher than control, but the result was however significantly lower in the dark portion of the L/D transition

box when compared to control. Chamber duration for light and dark portion in the L/D transition box for kola nut fed mice were however not significantly different from control. For the caffeine diet-fed mice, chamber duration for light portion of the L/D transition box was significantly higher when compared to control but significantly lower in the dark portion of the L/D transition box compared to control. This result however, did not differ significantly when compared to the kola nut fed mice. The frequency of stretch-attend postures in the light and dark portion of the L/D transition box for the kola nut and caffeine-fed mice were significantly lower when compared to their control respectively. Moreso, the duration of stretch-attend postures in both light and dark portions of the L/D transition box for kola nut and caffeine-fed mice were not significantly different.

The duration of entry into the open arm of the elevated plus maze for the kola nut and caffeine diet-fed mice were significantly higher when compared to their control respectively. There was however no significant difference in the duration of entry into the closed arm of the maze for the kola nut and caffeine diet-fed mice when compared to their control respectively. This therefore means that the kola nut and caffeine-fed mice were less fearful and less anxious when compared to their control group respectively. This is in line with the result obtained in the light/dark transition box in which the test mice spent more time in the light chamber of the L/D transition box. This result indicates that, the test mice were less fearful and less anxious when compared to the control.

In conclusion, chronic consumption of kola nuts diet and caffeine diets caused increased exploration and decreased anxiety and fear related behaviour in mice. These effects were more visible in the caffeine diet-fed mice. Therefore, the effect on kola nut diet-fed mice may not be due to caffeine action only. If these results are applicable to man, kola nuts and caffeine consumption could be used as additives in athlete's meal to enhance their performance. Also, they could be used to combat fear and anxiety related behaviours.

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