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Long-term Consumption of Kola-nut (*Cola nitida*) Diet Does Not Increase Anxiety Related Behaviour in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAB and EEO designed the study, author SAB performed the statistical analysis, wrote the protocol, author IOA wrote the first draft of the manuscript. Authors CCM and CON managed the analyses of the study. Authors SAB and IOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Following long-term consumption of kola nut (*Cola nitida)* diet, anxiety related behaviour were studied in 16 Swiss white mice (18-28g body weight). The open field (OF) test, elevated plus maze EPM) and the light/light transition box (LD) tests were used. Swiss white mice were fed either control diet (rodent chow; n=8) or kola nut diet (50% w/w kola-nut diet; n=8) for 28 days. All animals were allowed free access to clean drinking water. Results showed that the frequency of rearing in the kola nut diet group was lower ($p< 0.05$) compared to control. The non-exploratory behaviours like grooming and genital licking were also lower in the test group compared to control ($p<0.001$, 0.05 respectively). In the EPM test, the duration in the open arm in the kola diet group was higher compared to control ($p< 0.01$). The duration of grooming in the test group was however higher in the closed arm compared with control ($p< 0.01$). The frequency of downward dips only correlated positively with the duration in the open arm in the control [$r (16) = 0.855$; $p< 0.01$]. The

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kola fed animals spent more time in the light region of the LD test ($p< 0.01$) rearing and walling ($p<0.05$), and spent less time in the dark region when compared with their control. In conclusion, long-term consumption of kola nut diet decreased anxiety–related behaviour in the mice.

Keywords: Kola nut; anxiety; Swiss white mice.

1. INTRODUCTION

Kola is a tropical tree crop (family - *sterculiaceae*) which has socio-economic importance in Nigeria. Kola nut is used traditionally for ceremonies related to marriages, child naming, funerals and in other festivals and also chewed as a masticant [1]. 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. In Cross River and Akwa Ibom it is called "Ibong" in Efik language. It is of great importance in the traditional institution. In fact, the Igbos in Nigeria liken kola nut as a fruit that brings with it good fortunes and life.

Whichever way the nuts are consumed (chewed raw or used in powdered form), the nuts produce a mild stimulates effect on the central nervous system and thus producing a tentative feeling of increased energy and reduction of hunger and fatigue [2]. Fresh and cured kola nut chewed in small doses increase mental activity, reduce the need to sleep and also dispel hunger and thirst [1]. Therefore, kola nut chewing has become very popular among students, drivers and many other consumers who need to remain active for unusually long periods.

In some developed countries, however, kola nut extracts are used industrially for the manufacturing of many cola-type soft drinks flavours [3], as a source of caffeine for manufacture of many pharmaceutical products and essential oils [4], and as a main ingredient in production of heat-tolerant chocolate bars [5]. In addition, caffeine is known to be a fat burner and therefore beneficial in assisting weight loss [6]. As a result of the commercial importance of kola nuts, a lot of research work has been done on *Cola nitida*, the kola of commerce, in Nigeria [7]. Presently, the bulk of kola nuts being produced in Nigeria are either consumed fresh locally or exported as sun-dried to some drier areas of Africa, where they are used as masticant or as sources of colourant for cloth dyeing but with little or no industrial use locally [8].

There are over forty kola species but the most common species with major economic importance in Nigeria are *Cola nitida* and *Cola accuminata* [9]. Phytochemistry of *Cola nitida* show that it has 9.73-9.81% water, 2.72-2.21% ash, 3.02-2.20% fat, 19.14-15.24% protein, 7.30-4.18% crude fibre and 58.09 to 66.45% carbohydrate. *C. nitida* also has alkaloids (2.22%), tannin (6.46%) and saponin (8.06%), phenol (0.27%), flavonoid (0.12-0.14%) [10]. Caffeine is one of the most important alkaloids of *Cola nitida* and it forms 2.4% of its content [11].

Caffeine, a major alkaloid of *Cola nitida*, has been reported in some studies in mice to increase anxiety [12] while in other instances was reported to decreased anxiety [13]. In a human study on the effect of caffeine consumption among secondary school children in South West England, caffeine caused increased anxiety related behaviour [14].

Since kola nut contains caffeine, and there are still controversies as to whether chronic use of caffeine reduces or increases anxiety, this study saw the need to investigate the effect long-term consumption of kola nut on anxiety-related behaviour. Given the chronic consumption of kola nuts among locals in Nigeria, we studied the effect of long-term consumption of kola nut on locomotor activity and anxiety-related behaviour in CD1 mice using the open field test, elevated plus maze test and light dark transition test.

2. MATERIALS AND METHODS

Male Swiss white mice purchased from the animal house of the Department of Physiology, University of Calabar. Mice were kept in standard well ventilated animal facility in the Department of Physiology at temperature of 26±2ºC, 12/12 light/dark cycle. Mice were grouped into two: Control (n=8) given normal rodent chow (Vital feed Nigeria), and test (n=8) given 50% w/w kola-nut diet. Kola-nut diet was prepared by slicing, drying and grinding fresh kola-nut (*Cola nitida)* bought from Bogobiri (Hausa Market) in Calabar, Nigeria. Equal portions of the grinded kola-nut powder (10g) and rodent chow (10g) were used to constitute 50% w/w kola-nut diet [15]. All animals had access to clean drinking water and food *ad libitum*. This feeding was done for 28 days. All animals were weighed before and after the feeding period.

Internationally acceptable ethics for laboratory animal used were strictly adhered to. Ethical approval was duly obtained from the Faculty of Basic Medical Sciences Animal Ethics Committee with Protocol number 014PHS015007

The open field apparatus test used by Bisong et al (2006, 2018) was employed in this study. The test apparatus measured 72 x 72 x 32cm (l x b x h) with a floor divided into sixteen 18 x 18cm squares, and centre square of 36 x 36cm. Mice were allowed 5 minutes to explore the apparatus while behaviour are scored. Thirty minutes later, mice were tested for another 5 minutes [16].

The elevated plus maze as described by Lister [17] and used by Bisong et al. [18,19] was employed in this study. The apparatus has 2 open arms (each measuring 30x5x15cm) and two closed arms (each measuring 30x5x15cm) extending from a central, open square (5x5cm). The maze was elevated on a pedestal to a height of 45cm above the floor. Each mouse was placed in the centre square of the elevated plus maze facing an open arm and its behaviour scored.

The light/dark transition box test as described by Hascoët and Bourini [20] and used by Bisong et al. [18] was used. It is box measuring 46x30x27cm high (l x b x h cm), divided into two compartments; a small 18x30cm area and a large 27x30cm area with a 7x7cm door on floor of the partition linking the two chambers. The small compartment was painted black to mimic darkness, whereas the large compartment was painted white. Mice were given 5 minutes to explore the apparatus during which behaviours were scored.

2.1 Statistical Analysis

Data obtained from the study were analysed using the Student T- test. Associations between data were tested using the Pearson's correlation. Data were presented as means ± standard error of mean. Probability level of $P< 0.05$ was accepted as significant.

3. RESULTS

3.1 Exploratory Behaviour in the Open Field Test

The Exploratory behaviour**,** Rearing and walling, are forms of vertical locomotor activity. The frequency of Rearing in the test group within the first five minutes was 3.6 ± 1.1/5min, significantly lower when compared with control (5.9 ± 1.5/5min; $P< 0.05$). At the end of 30 minutes, the frequency of rearing in the kola group (43.4 ± 11.1/30min) was also lower than that in control (76.4 ± 11.2/30min; $P< 0.05$). Although the frequency of walling in the kola group seemed lower both at 5 minutes (16.4 ± 3.6/5min) and 30 minutes (58.3 ±14.0/30min) when compared with control (19.6 ± 2.3 at 5 minutes and 80.4 ± 10.7 at 30minutes; Fig. 1) it did not differ significantly.

3.2 Grooming in the Open Fields Test

The frequency and duration of grooming in the open field apparatus were significantly lower in the test group than in the control at the end of the 30 minutes session. This was 16.4 ± 2.8/30min in the kola group, which was less than that in the control group of animals, which was 30.1 ± 2.9; ($P< 0.01$). The duration of the grooming at the end of 30minutes (192.5 ± 25.4 sec) was also significantly lower in the kola fed group compared to control (352 ± 54 sec; $P< 0.05$). This is shown in Fig. 2.

3.3 Comparison of Activities in the Elevated plus Maze between Kola Fed and Control Mice

The frequency of entry into the open arm was not significantly different between control and kola fed mice. The duration in the open arm was however, significantly higher in the test group compared to control ($p < 0.01$). The frequency and duration of downward dipping of mice did not differ significantly between test and control groups of mice. The frequency of entry into the closed arm of the elevated plus maze in the kola fed group was significantly lower compared to control ($P< 0.05$). The duration in the closed arm was also lower in the test group compared to control ($P< 0.05$). Although the frequency of grooming in the closed arm was not significantly different between test and control groups, the duration differed ($P<0.01$). The frequency of rearing and the number of faecal boli at the end of the 5minutes session did not differ from control values. Table 1 shows a summary of these comparisons.

3.4 Correlation between Duration in the Open Arm and Frequency of Downward Head Dipping

There was a positive correlation between the duration in the open arm of the elevated plus maze and the frequency of downward head dipping in the control group [$r (16) = 0.855$; $p< 0.01$], Fig. 3.

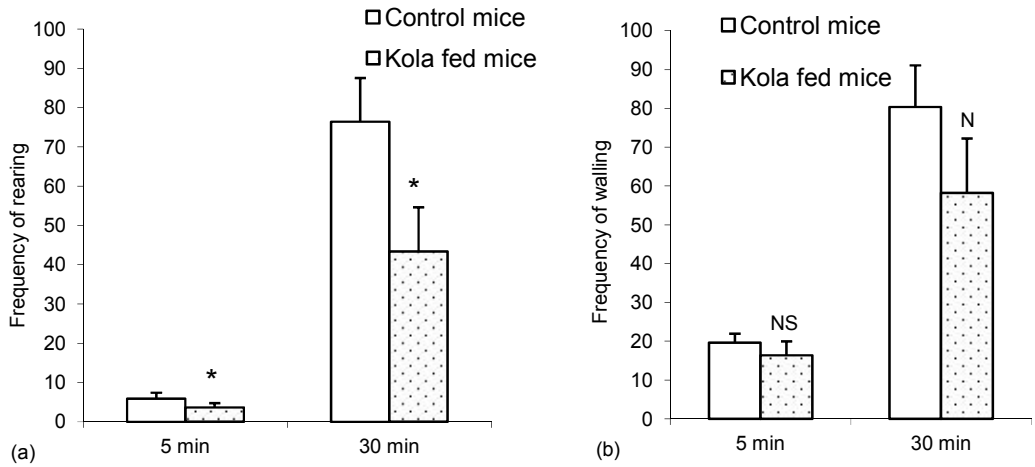

Fig. 1. Exploratory behaviour; rearing (a) and walling (b) of mice fed kola diet in the open field test

NS – Not significant compared to control
** - Significant at P< 0.05 compared to control*

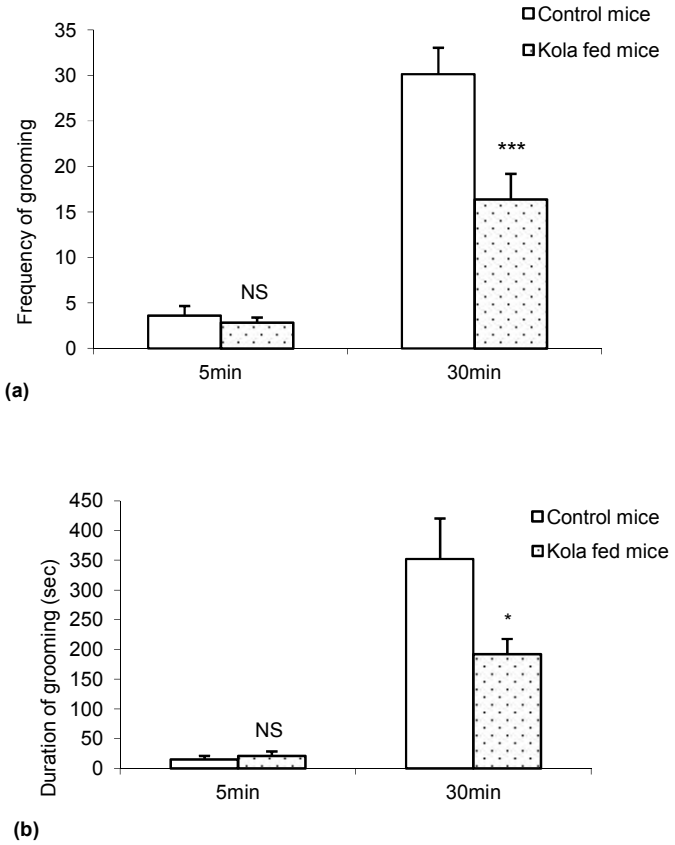

Fig. 2. Frequency (a) and Duration (b) of grooming in mice fed kola diet in five minutes and thirty minutes in the open field test

NS – Not significant compared to control
** - Significant at P< 0.05 compared to control.*

Table 1. Summary of comparison of activities in the Elevated plus maze between kola fed and control mice

Parameters	Control group	Kola fed group	Level of significance
Frequency of open arm entry (/5minutes)	2.8 ± 0.3	2.6 ± 0.6	NS
Duration in open arm (seconds)	45.3 ± 10.4	136 ± 18.7	0.01
Frequency of downward head dipping (/5minutes)	11.4 ± 2.9	12 ± 2.2	NS
Duration in closed arm (seconds)	218.1 ± 15.2	158.9 ± 25.5	0.05
Frequency of grooming in the closed arm (/5 minutes)	3.3 ± 0.8	4 ± 0.5	NS
Duration of grooming in the closed arm (Seconds)	18.3 ± 5.7	44.9 ± 9.3	0.01
Frequency of genital licking in the closed arm (/5 minutes)	1.3 ± 0.4	2.3 ± 0.5	0.05
Frequency of rearing in the closed arm	19 ± 2.0	20 ± 2.1	NS
Number of faecal boli	0.4 ± 0.3	0.3 ± 0.2	NS

NS – Not significantly different compared to control

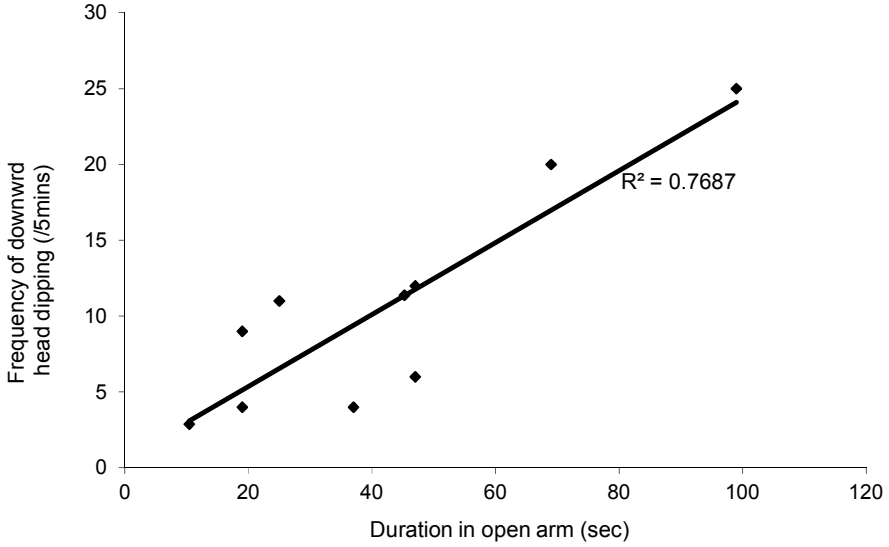

Fig. 3. Correlation between frequency of downward head dipping and duration in the open arm in the control group

3.5 The Effect of Long-term Feeding with Kola Diet on Activities in the Light Region of the Light/ Dark Transition Box

Activities in the light region were generally higher for the kola fed group compared to control. Although the frequency of entry into the light region did not differ, the time spent (duration) in the light region was significantly higher in the test group (111.5 ± 9.43 seconds) compared to control (77.63 ± 12.7 seconds; $p< 0.05$). The frequency of line crossing did not differ between the test and control groups. The frequency of rearing and walling were higher in the test groups compared to control ($p< 0.01$; $p<0.05$ respectively), Table 2.

3.6 The Effect of Chronic Feeding with Kola Diet on Activities in the Dark Region of the Light/ Dark Transition Box

Although the test group showed less activity in the dark region of the light/dark transition box, the exploratory activities were significantly higher compared to control. The frequency of entry and the time spent (duration) in the dark region did not differ in the test group when compared to control. Frequency of rearing in the test group was higher than the value for the control group ($p< 0.05$). Walling also followed a similar trend with the test group of animals walling more than control ($p<0.05$). The frequency of grooming did not differ significantly but the duration of

Table 2. Activities in the light region of the light/ dark transition box

Activity in the light region.	Control group	Kola fed group	Level of significance
Frequency of entry (/5mins)	6.75 ± 0.90	6.0 ± 0.95	NS
Duration in light region (Sec)	77.63 ± 12.7	111.5 ± 9.43	0.05
Line crossing (/5mins)	59.0 ± 10.29	51.3 ± 6.43	NS
Frequency of rearing (/5mins)	3.13 ± 0.82	9.25 ± 1.65	0.01
Frequency of walling (/5mins)	7.75 ± 1.75	14.88 ± 2.76	0.05

NS – not significant compared to control

Table 3. Activities in the dark region of the light/ dark transition box

Activity in the dark region.	Control group	Kola fed group	Level of significance
Frequency of entry (/5mins)	7.75 ± 0.90	5.13 ± 0.83	NS
Duration in dark region (Sec)	222.5 ± 12.37	197 ± 13.97	NS
Frequency of rearing (/5mins)	8.5 ± 1.56	14.0 ± 3.1	0.05
Frequency of walling (/5mins)	12.88 ± 2.53	20.4 ± 2.74	0.05
Frequency of grooming (/5mins)	6.38 ± 6.0	6.0 ± 1.12	NS
Duration of grooming (sec)	63.88 ± 13.73	32.6 ± 6.43	0.05
Frequency of genital licking (/5mins)	4.5 ± 1.09	2.0 ± 0.61	0.05

NS – not significant compared to control

grooming was lower in the test group compared to control ($p< 0.05$). The frequency of genital licking in the test group of mice was also significantly lower compared to that in the control group ($p< 0.05$); Table 3.

4. DISCUSSION AND CONCLUSION

The open field apparatus has been used to assess the emotionality of animals in a novel environment, as well as locomotion and exploration [21]. Although the frequency of line crossing did not differ significantly between the test and control groups, other forms of locomotor activity differed. This implies that there was no significant change in horizontal locomotor patterns.

The frequency of rearing was significantly lower in the kola fed group (test) when compared to control in the open field. This trend was also similar in the frequency of walling. The vertical locomotor activities and therefore exploratory activity was decreased following long-term ingestion of kola nut. The frequency and duration of grooming in the kola fed group were also significantly decreased at the end of 30 minutes. This implies decreased vertical locomotor (exploratory) activities following chronic ingestion of kola nut. Therefore long-term ingestion of 50%w/w kola nut diet decreased exploratory activity. It is most likely that consumption of large quantities of cola nut will not serve the stimulant effect any longer but rather cause depression of the nervous system. These results however, do not support the report of previous researchers that kola nuts serve as a stimulant [1,2]. The decrease in exploratory/vertical locomotor activity following long-term consumption of kola is in consonance with reports of Neil [22] which showed that excessive consumption of caffeine caused mixed depressive states in psychiatric patients; and also the work of Greden [23] depressive syndrome as being associated with caffeine, which is one of the major constituents of kola nut.

The elevated plus maze has been proven as a model for assessing anxiety and fear [16,17]. This test is based on the natural aversion of rodents for open space and heights. Mice fed 50%w/w kola diet when compared with control, spent more time in the open arm and less time in the closed arm. Since the open arm is the aversive arm, drugs that reduce anxiety would thus cause the animals to spend more time in the open arms of the maze. Therefore, that the kola-fed mice were less fearful compared to their control. To buttress these, there was a positive correction between the duration in open arm and the frequency of downward head dipping, as mice which are less fearful would perform more head dips. Therefore, the kola diet reduced anxiety in the mice.

The light/dark transition box is also used as a model for assessing anxiety and fear. This light/dark test is based on the innate aversion of rodents for brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors that is novel environment and light [24]. Mice which are less anxious would spend more time int the open space and brightly lit chamber. In this test, the kola fed group of mice spent more time in the illuminated (light) region of the box and also showed more activity (rearing and walling) in this region. This implies that long-term feeding of mice with kola diet produces an anxiolytic effect.

The kola fed group showed less grooming (non-exploratory behaviours associated with fear) in the dark region compared to their control. The frequency of the exploratory behaviours, rearing and walling, were higher in the test group. This is in agreement with findings of Costal et al. [25] that increased exploratory behaviours was associated with an increase in the time spent in the light region of the light/ dark box. Grooming is a displacement reaction which happens when mice are anxious. Thus increased grooming behaviour indicate increased anxiety.

The mice in the test group were less fearful compared to control, which is in consonance with the test in the elevated plus maze. Therefore, long-term ingestion kola diet caused an anxiolytic effect. These results were contrary to earlier works which implicate caffeine (a major component of kola) as an anxiogenic agent [26,27].

In conclusion, long-term consumption of 50%w/w kola diet in mice caused decreased exploratory activity, instead of producing a stimulant effect, in mice. The kola diet also produced an anxiolytic effect, thereby reducing fear and anxiety in mice.

ETHICAL APPROVAL

Internationally acceptable ethics for laboratory animal used were strictly adhered to. Ethical approval was duly obtained from the Faculty of Basic Medical Sciences Animal Ethics Committee with Protocol number 014PHS015007.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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