



## Fear and Anxiety Behaviour In Mice Was Attenuated Following Chronic Consumption of *Musa paradisiaca* L. (Musaceae)

Peter Erigbali<sup>1\*</sup>, Eme Osim<sup>2</sup>, Ofem Ofem<sup>2</sup><sup>1</sup>Department of Physiology, Niger Delta University, Amassoma, Bayelsa State, Nigeria<sup>2</sup>Department of Physiology, University of Calabar, Calabar, Nigeria

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## ABSTRACT

Emotions are delicate phenomenon that can affect the whole livelihood, yet to control them physiologically is quite difficult perhaps owing to the paucity in connections between limbic system and neocortex. Many people have devised ways such as consumption of alcohol and hard drugs, to help them control their emotions. Research findings have shown the link between diet and some emotions/neurobehaviour. We investigated the effect of consumption of unripe plantain on fear and anxiety behaviour. Three groups of mice were fed with diet containing 100%, 50% and 0% (control) unripe plantain. Fear and anxiety behaviour was studied using the Light/Dark Transition Box and Elevated Plus Maze tests. The transitions in the light/dark chambers was not significantly ( $p > 0.05$ ) different among the groups. The light chamber duration of the 100% and 50% plantain diet groups was significantly ( $p < 0.05$ ) higher than control. Stretch attend postures were lower in 100% and 50% plantain diet groups compared to control. In the Elevated Plus Maze test, frequencies of open arm entry for the 100% and 50% plantain groups were significantly ( $p < 0.05$ ) higher than control. The difference in open arm entries between 100% and 50% plantain diet groups was significant. The results suggest that consumption of plantain diet reduced fear and anxiety behaviour in mice. A preliminary investigation of the concentration of serotonin in the brains of the mice using High Performance Liquid Chromatography shows that serotonin is significantly ( $p < 0.001$ ) higher in the 100% plantain diet group than control.

**Keywords:** Plantain, Serotonin, 5-Hydroxytryptophan, Fear, Anxiety.

## Introduction

Human behaviour is believed to be influenced by the endocrine and nervous systems, and the complexity in the behaviour of an organism is correlated to the complexity of its nervous system. Thus, organisms with more complex nervous systems, like humans have a greater capacity to learn new responses and adjust their behaviour. This behaviour is influenced by physical and psychological changes that result from a complex state of feeling described as emotion.<sup>1,2</sup> Meanwhile, paucity of connections between the limbic system (the part of the brain that controls our emotion) and neocortex (the part of the brain whose activity can modify emotional behaviour) as well as the prolonged after discharge of the limbic system after stimulation have made it physiologically difficult to bring our emotions under control.

Many people have explored various methods such as music, yoga, exercise and religion all of which are believed to affect emotional state in one way or the other. According to one study, some music cause positive emotion like happiness, some cause negative emotion like sadness and fear, but loud music could cause deafness. Others in the quest to ensure a healthy emotional state have resorted to consumption of alcohol and hard drugs and these have their attendant demerits. Alcohol is the most popular anti-

depressant drug used by mankind. It also decreases anxiety and boosts confidence. But it has several side effects. For instance, alcohol affects the limbic system; the person is subject to exaggerated states of emotion (anger, aggressiveness, withdrawal and memory loss).

Drugs target the limbic system to induce positive emotion, by acting in the nucleus acumbens and the reward areas of the limbic system, but they block a few of the negative emotions which act as defences for the individual.<sup>3,4</sup>

Emerging research evidence from exploration of dietary consumables are suggesting correlation between choice of diet and emotion or other neurobehaviour. It has been reported that a staggering 20.9 million Americans suffer from mood disorders that may be linked to dietary choices.<sup>5</sup> Some researchers are already exploring the effect of poor diet on the mind; while others are searching for straightforward answers to emotional woes. For instance, less occurrence of depression in a research population that consumed Omega-3 fat and alteration of brain chemistry, leading to more balanced, clear and joyful mental states associated with a varied, nutrient dense diet have been reported.<sup>5,6</sup>

*Musa paradisiaca*, commonly called plantain is one of the over forty species of the genus *musa*. This food crop is generally eaten cooked, fried, roasted ripe or unripe to the soft, sweet banana, which is of the same genus but eaten raw when ripe. Plantain is affordable and readily available all year round in areas where it is cultivated. In equatorial Africa, Andean regions and many countries of the world, it is a staple diet. Plantain is rich in carbohydrates and fiber but lacks cholesterol. It contains vitamins A, B<sub>6</sub>, C and minerals; potassium, magnesium among others.<sup>7,8</sup> Besides the nutritional constituent, plantain contains some neurotransmitters, notably serotonin and its precursor, 5-Hydroxytryptophan that can affect emotion/mood.<sup>9</sup> It is known that the medical management of emotional disorders is a cumbersome process that involves the use of expensive antipsychotic drugs, many of which when

\*Corresponding author. E mail: [perigbali@yahoo.com](mailto:perigbali@yahoo.com)  
Tel: +2348033258343

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affordable, have side effects such as blurred vision, dry mouth, drowsiness, muscle spasms or tremors and rapid weight gain.<sup>10</sup> This research was therefore carried out to see if staple foods that provide nourishment and have fewer side effects than drugs can contribute to the management of emotional problems. The study investigated the effect of long-term consumption of unripe plantain diet on fear and anxiety-related behaviours in mice.

## Materials and Methods

Thirty adult male Swiss mice were used for the study; following due approval by ethical committee on Research with animals and compliance with international guidelines and regulations of experiment involving animals. Mice were housed in single cages, under controlled room temperature ( $28 \pm 2^\circ\text{C}$ ) and humidity ( $85 \pm 5\%$ ). The animals were kept in a normal light/dark cycle and allowed to acclimatize for 2 weeks before any experiment started. All animals had access to rodent chow and clean drinking water *ad libitum*. At the start of the experiment, the mice were placed in three groups of ten that were fed either 100% plantain diet (100% plantain diet group), 50% plantain diet (mixed diet group) or normal rodent chow (control group). The feedings lasted for 30 days before fear and anxiety studies were done using the Light/Dark Transition box and Elevated plus Maze tests.

### Preparation of Plantain Diet

Bunch of plantains was purchased from the central market in Calabar, Nigeria. The peels were removed and the pulp washed, chopped into slices and oven dried at  $40^\circ\text{C}$  and 55% humidity for 24 h. The dehydrated slices were then ground into powder in a grinding mill, in line with the previous method.<sup>11</sup> This was the 100% plantain diet. The 50% plantain diet (mixed diet) was prepared from plantain powder (flour) by measuring equal gram weights of plantain flour and rodent chow and mixing thoroughly.

### The light/dark transition box Test

This was used to assess anxiety and fear-related behaviours in the groups of mice. The light and dark transition box (LD Box) constitutes a test that assesses an unconditioned anxiety and exploratory behaviours. It is based on the perceived conflict in mice between exploring in a novel environment and avoidance of bright light.<sup>12,13</sup> Mice were scooped up using a plastic container, placed in the center of the white compartment/chamber of the LD Box, facing the opening (door) that links the light and dark chambers and allowed to explore the apparatus for 5 min, while the behaviours of the animals were recorded using a camcorder. After 5 min, mice were removed from the light and dark box by the base of their tails and returned to their home cages. The behaviours recorded include; transitions of mice in the compartments, duration in the light chamber and stretch attend postures.

### The Elevated plus maze (EPM)

The EPM test is based on the inborn aversion of rodents for open, bright illuminated spaces. The maze consisted of two open arms ( $30 \times 5$  cm) and two closed arms ( $30 \times 5$  cm) that were enclosed by a sidewall on all edges (height 15 cm). Mice were scooped in a plastic container and placed in the center of the maze (central platform) facing the closed arm. The frequency and duration of open arm and closed arm entries were quantified for a 5-minutes test. Arm entry was only defined when an animal (the mouse mass center) was at least 3 cm on an arm to differentiate entries from stretched attend postures into the arms.<sup>14</sup>

### Neurochemical Analysis

Established method<sup>15</sup> was used to estimate the concentration of serotonin in the brains of all the mice as follows; Animals were anaesthetized with ethyl chloride; brains were removed, weighed and snap frozen on dry ice. The frozen tissues were homogenized in lyses buffer (containing 10  $\mu\text{M}$  ascorbic acid and 18% perchloric acid); centrifuged for 30 min at 20,000 xg,  $4^\circ\text{C}$  and the supernatant was used for HPLC analysis. Sample separation occurs at  $20^\circ\text{C}$  on C-18 Reversed-phase column using a 10  $\mu\text{M}$  potassium phosphate buffer, pH 5.0, containing 5% methanol and at a flow rate of 2 mL/min. Fluorescence of 5-Hydroxytryptophan (5-HTP) and serotonin (5-HT) is excited at 295 nm and measured at 345 nm. Amount of 5-HTP and 5-HT were normalized to wet tissue weight for statistical analysis and calculation of substance levels was based on external standard values.

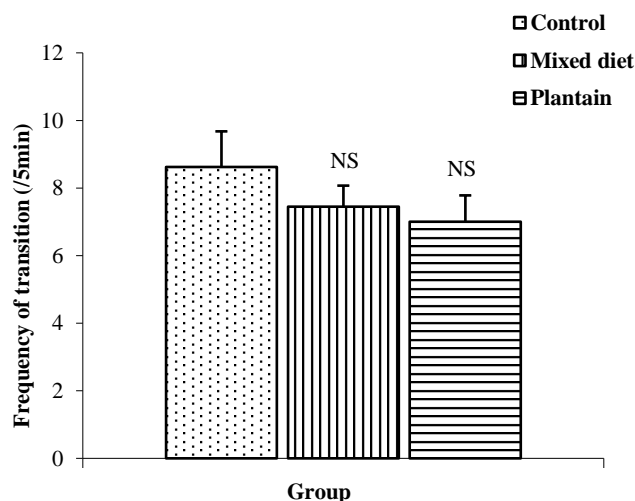
## Results and Discussion

The physiological basis of an LD Box test in mice is focused on the conflict between exploring in a novel environment and avoidance of bright light. Normally, mice placed in the light section which is the aversive chamber should move quickly to the dark section, while mice placed in the dark section should show hesitation before entering the light section. Also, mice spend more time in the dark chamber of the apparatus. Thus, the greater amount of time spent in the light chamber implies decreased fear-related behaviour.

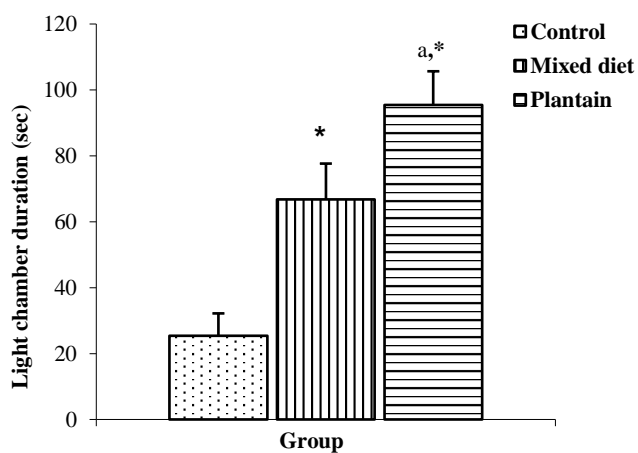
In the LD box, the transition of all groups of mice across the light and dark chambers was not significant at  $p < 0.05$  as shown in figure 1, implying that all the mice had equal chances of being in either the dark or light chamber of the box. Figure 2 indicates that the light chamber duration of the 100% and 50% plantain diet groups was significantly ( $p < 0.05$ ) higher than control, suggesting that the plantain diet-fed mice showed less fear and anxiety; as they spent more time in the light chamber. Also, the duration of 100% plantain group in the light chamber was higher than the 50%, which may be indicative of a dose-dependent effect of the plantain diet. Stretch attend postures are risk assessment behaviours which indicate that the animal is hesitant to move from its present location to a new position.<sup>16</sup> Thus, a lower frequency of this behaviour indicates a lower degree of anxiety. In figure 3, the results show that stretch attend posture was significantly decreased in both 100% and 50% plantain diet-fed mice than control.

The assessment of fear and anxiety in the elevated plus maze in figure 4 shows that the frequency of open arm entry was significantly higher in the two groups that consumed plantain (100% and 50%), indicating less fear and anxiety compared to control group that stayed more in the closed arm. Similarly, the open arm duration of the 100% and 50% plantain groups was significantly ( $p < 0.05$ ) higher than control (figure 5). This effect may also be dose-dependent since the 100% plantain groups showed higher open arm entry and duration than the 50% group. The implication being that more quantity of plantain may have reduced fear and anxiety behaviour to a greater extent. This supports the observations in the LD Box test.

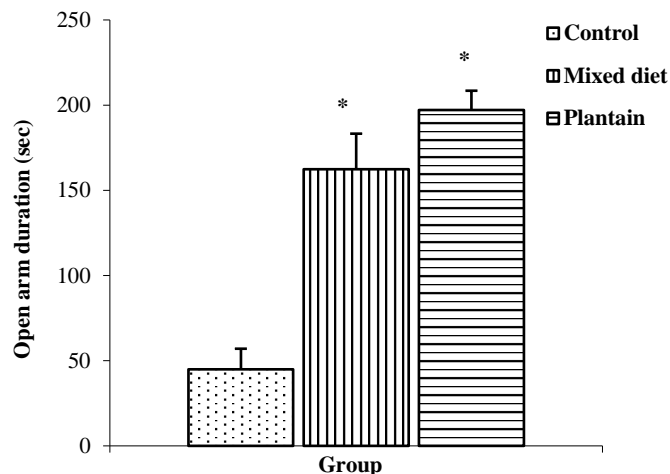
A preliminary neurochemical analysis with the brains of all three groups of mice to estimate concentration of a possible active ingredient (serotonin) in plantain, using High-Performance Liquid Chromatography shows that serotonin was significantly ( $p < 0.001$ ) higher in the 100% plantain diet group than control and 50% groups; also serotonin was higher in the 50% plantain group than control but not significantly as shown in figure 6.



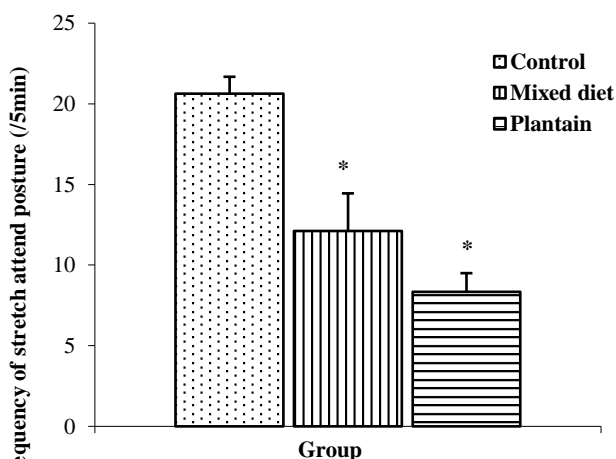
**Figure 1:** Comparison of frequency of transitions in the light and dark box in the different experimental groups. Values are mean + SEM,  $n = 9$ .  $p < 0.05$ , NS = Not significant.



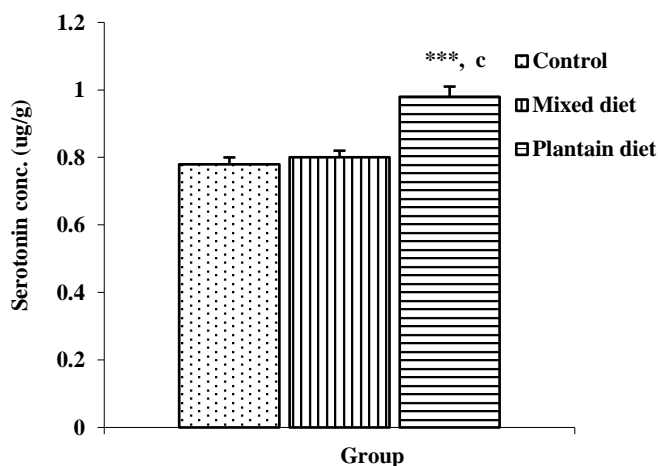
**Figure 2:** Comparison of light chamber duration in the light and dark transition box test in the different experimental groups. Values are mean + SEM, n = 9. \*p < 0.05 vs control; a = p < 0.05 vs mixed diet.



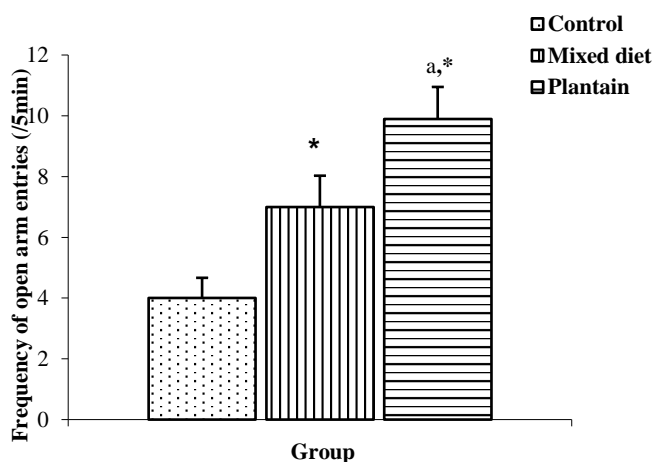
**Figure 5:** Comparison of open arm duration in the Elevated plus maze in the different experimental groups. Values are mean + SEM, n = 9. \*p < 0.05 vs control.



**Figure 3:** Comparison of frequency of stretch attend postures in the light and dark transition box in the different experimental groups. Values are mean + SEM, n = 9. \*p < 0.05 vs control.



**Figure 6:** Comparison of brain serotonin concentrations in the different experimental groups. Values are mean + SEM. \*\*\*p < 0.001 vs control; c = p < 0.001 vs mixed diet.



**Figure 4:** Comparison of open arm entry in the Elevated plus maze in the different experimental groups. Values are mean + SEM, n = 9. \*p < 0.05 vs control; a = p < 0.05 vs mixed diet.

The observed higher concentration of serotonin in the brains of the mice that consumed either doses/quantity of plantain may be an indication that plantain probably increased the level of serotonin in the brains of those mice. And if this was the case, the observations may corroborate the assertion that serotonin neural circuits frequently serve to counterbalance the arousing activating dopamine/noradrenaline circuit, so that anxious, agitated emotion occurs when a person's dopamine/noradrenaline activating arousal circuits are functioning strongly, without the calming, relaxing, mellowing serotonin circuits functioning strongly as a complementary counter balance.<sup>17</sup> It is possible that the plantain diet-fed mice showed less anxiety and fear related behaviours because plantain increased the level of brain serotonin which may have facilitated the calming, relaxing and mellowing serotonin circuits.

### Conclusion

This study suggests that long-term consumption of unripe plantain diet decreased fear and anxiety behaviour in mice. Further research to elucidate the mechanism will be worthwhile.

### Conflict of interest

The authors declare no conflict of interest.

**Authors' declaration**

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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