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*Afr. J. Biomed. Res. Vol. 20 (September, 2017); 321- 323*

*Short communication*

## **Effects of Diets Containing Unripe Plantain Diet on Brain Serotonin in Mice**

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### **ABSTRACT**

*Musa paradisiaca* (Plantain) fruit has been shown to be useful for nutritional, medicinal and industrial purposes. It contains serotonin (5-HT) and its immediate precursor, 5-Hydroxytryptophan (5-HTP). Serotonin plays a fundamental role in integration of behaviour and many physiological functions including regulation of mood, anxiety, arousal, aggression, impulse control, and thinking abilities. In this study, the effect of plantain-containing mouse diet on brain serotonin mice was investigated in mice. Thirty adult Swiss mice were divided into three groups of ten each and fed normal rodent chow containing 0%, 50% and 100% unripe plantain. After thirty days, the brain levels of 5-HT and 5-HTP were measured using High Performance Liquid Chromatography (HPLC) technique. Results show that 5-HT level was significantly increased in the plantain-diet fed mice. Conversely, 5-HTP level was reduced in the 100% plantain fed mice when compared with the control.

**Keywords:** *Musa paradisiaca*, Unripe plantain, serotonin, 5-Hydroxytryptophan

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*Received: February, 2017; Revised version accepted: August, 2017*

### **Abstracted by:**

*Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius*

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### **INTRODUCTION**

*Musa paradisiaca*, commonly called plantain is a vastly cultivated food crop that constitutes a staple diet in the food of many populations. Plantain is rich in carbohydrates and fiber but lacks cholesterol. It contains vitamins A, B6, C and minerals; potassium, magnesium among others (Osim et al., 1990; Lheureux et al., 2007). It contains some neurotransmitters including, dopamine, noradrenaline, adrenaline, tyramine, octopamine and serotonin. In the body, serotonin is synthesized from the amino acid tryptophan which is converted into 5-HTP by tryptophan hydroxylase. 5-HTP is then converted by aromatic amino acid decarboxylase into serotonin (Portas et al., 2000; Walther et al., 2003; Daniel & Michael, 2007). 5-HT in the central nervous system is principally synthesized by neurons of the raphe nuclei whose axons extend throughout the brain. Notably, plantain also contains tryptophan and 5-HTP. It is long established that serotonin does not cross the blood brain barrier, while its precursors, 5-HTP and tryptophan easily cross the blood brain barrier (Young & Teff, 1989). However, studies to re-evaluate the notion that serotonin does not cross the blood brain barrier have shown that augmented brain serotonin crosses the blood

brain barrier through the 5-HT transporter (Nakatani et al., 2008).

Serotonin is a widely studied neurotransmitter that helps regulate a vast range of psychological and biological functions. It is involved in regulation of mood, anxiety, arousal, aggression, impulse control, and thinking abilities. Other brain chemicals, such as dopamine and norepinephrine, also influence mood and arousal. However, disruption of the normal functioning of the serotonin system may often occur leading to serious anomalies such as serotonin deficiency syndrome which has been shown to manifest as a broad array of emotional and behavioural problems. They range from depression, anxiety, alcoholism, insomnia, violence, aggression, suicide, compulsive gambling, sexual aberrations, obesity or eating disorders and chronic pain (Wang & Yan, 2002). This problem is caused by a chronic deficit of serotonin in the nerves that use it as their neurotransmitter, usually due to problems relating to the nutritional biochemistry of tryptophan (Young & Teff, 1989).

The management of many of these disorders is cumbersome and involves the use of expensive antipsychotic drugs, many of which when affordable, have side effects such as blurred vision, dry mouth, drowsiness, muscle spasms or

tremors and rapid weight gain, to mention a few (Muench & Hamer, 2010).

The objective of this study is to determine the levels of serotonin in the brain of mice fed with diets containing different proportions of unripe plantain.

## MATERIALS AND METHODS

**Preparation of animals:** Thirty adult Swiss albino mice were used for the study. They were purchased from the animal house in the department of Pharmacology, University of Calabar, Nigeria. The mice were randomly placed in three groups of ten that were housed singly in 28cm by 12cms by 16cm cages, under control room temperature ( $28 \pm 2^\circ\text{C}$ ). The animal room was properly ventilated and animals were kept in a normal light/dark cycle. They were allowed access to standard rodent chow (Grower's mash, vital feed company, Calabar, Nigeria) and clean drinking water ad libitum for 2weeks during acclimatization period prior to the study.

**Preparation of plantain diets:** Unripe plantain was purchased from a local market in Calabar, Nigeria. Hundred percent (100%) plantain diet was prepared according to the method of Delahaye (2008). The peels were removed and the pulp was rinsed and chopped into slices. The slices were oven dried at  $40^\circ\text{C}$  and 55 % humidity for 24 hours. Then the chips were ground into powder form (plantain flour). The flour was preserved in air tight plastic bowl from which they were served daily. Fifty percent (50%) plantain diet was prepared using the plantain flour (diet) and the normal rodent chow. Equal quantities were measured out from the plantain flour and the rodent chow and mixed properly in a 50/50 proportion to have the 50% plantain diet (mixed diet).

**Treatment groups:** Two categories of treatment diet were used in the study. The control diet that was the normal rodent chow was fed to the control group. Two treatment diets that contained 100% plantain and 50% plantain were fed to the 100% and 50% plantain diet groups. The mice were fed with the respective diet and clean water for 30 days.

**Biochemical Analysis:** The concentration of 5-HTP and 5-HT were measured in plantain using the methods of Feldman & M'lee (1985) and Mosienko et al (2012). Six Samples of unripe plantains were processed according to the method of Delahaye (2008) into plantain flour as indicated in the method of preparation of the hundred percent (100%) plantain diet. Each was homogenized on lyses buffer ( $10\mu\text{M}$  ascorbic acid and 18% perchloric acid); centrifuged for 30minutes at  $20,000\text{g}$ ,  $4^\circ\text{C}$  and the supernatant was used for HPLC analysis. Sample separation occur at  $20^\circ\text{C}$  on C18 reverse-phase column using a  $10\mu\text{M}$  potassium phosphate buffer, pH 5.0, containing 5% methanol at a flow rate of 2ml/min. Fluorescence of 5-HTP and 5-HT is excited at 295nm and measured at 345nm. Calculation of amount of 5-HTP and 5-HT was based on external standard values.

**Neurochemical Analysis:** At the end of the 30 days of feeding, the animals were anesthetized with ethyl chloride. Brains were removed, weighed and snap frozen on dry ice.

Frozen tissues were homogenized on lyses buffer ( $10\mu\text{M}$  ascorbic acid and 18% perchloric acid); centrifuged for 30minutes at  $20,000\text{g}$ ,  $4^\circ\text{C}$  and the supernatant was used for High Performance Liquid Chromatography (HPLC) analysis to measure the concentration of 5-HTP and 5-HT in the brains of the mice. This was in line with the method of Mosienko et al (2012).

Sample separation occurred at  $20^\circ\text{C}$  on C18 reverse-phase column using a  $10\mu\text{M}$  potassium phosphate buffer, pH 5.0, containing 5% methanol at a flow rate of 2ml/ min. Fluorescence of 5-HTP and 5-HT is excited at 295nm and measured at 345nm. 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. Standard 5-HTP and 5-HT were purchased from United States of America, 16144 Westwoods Blus Park Ellisville.

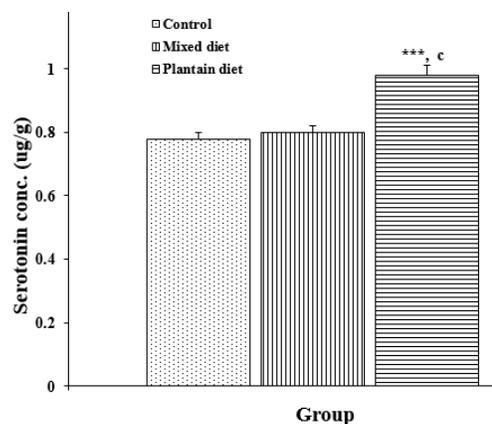
**Statistical Analysis:** The variance within and among samples of three groups were analyzed using one-way analysis of variance (ANOVA), while the statistical difference between groups of two were analyzed by post-hoc least significance difference (LSD) test at  $p < 0.001$ .

## RESULTS

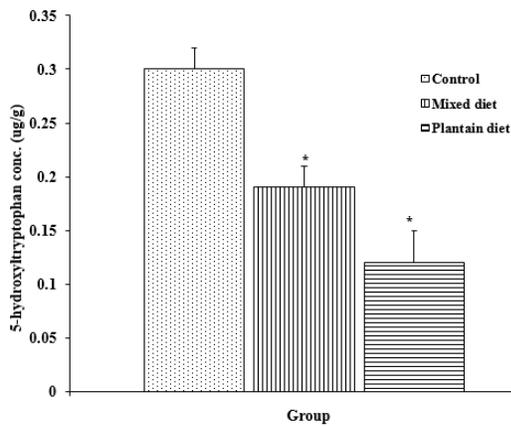
The result of the biochemical analysis showed that the concentration of serotonin in plantain was  $30.55 \pm 0.72 \mu\text{g/g}$ , while 5-HTP was  $1.03 \pm 0.07 \mu\text{g/g}$  as shown in Table 1. Fig. 1 shows that serotonin was significantly ( $p < 0.001$ ) higher in the 100% plantain fed-mice than the 50% and control groups. Meanwhile the concentration of 5-HTP in the brains was significantly ( $p < 0.05$ ) lower in the 100% plantain fed-mice than control (Fig. 2).

**Table 1**  
Concentration of 5-HTP and 5-HT in plantain

Samples	5-Hydroxytryptophan ( $\mu\text{g/g}$ )	Serotonin ( $\mu\text{g/g}$ )
Mean	$1.03 \pm 0.07$	$30.55 \pm 0.72$



**Figure 1**  
Comparison of brain serotonin concentrations in the different experimental groups. Values are mean  $\pm$  SEM. \*\*\* $p < 0.001$  vs control; c =  $p < 0.001$  vs mixed diet.



**Figure 2**

Comparison of 5-Hydroxytryptophan concentrations in the brains of the different experimental groups. Values are mean + SEM. \* $p < 0.05$  vs control

## DISCUSSION

Chronic consumption of unripe plantain in the dietary regimen of mice appears to contribute significantly to the levels of serotonin in the brains of mice. Although it is established that serotonin does not cross the blood brain barrier, its precursors easily do (Young & Teff, 1989). When mice were fed only plantain diet for thirty consecutive days, the levels of serotonin in their brains was increased compared to the control group. The concentration of the immediate precursor (5-HTP) of serotonin was reduced in the brains of the plantain fed-mice. It is possible that the precursors of serotonin that easily cross the blood brain barrier were responsible to facilitate biosynthesis of serotonin in the brains of the plantain fed-mice (Walther et al., 2003). This inference may be supported since the immediate precursor (5-HTP in particular) of serotonin was reduced in this study.

Perhaps other factors may have been actively involved in the observed trend. For instance, it is documented that plantain contains significant amounts of vitamin B6, which has been reported to act as co-factor in the conversion of 5-HTP into 5-HT, the rate limiting step in serotonin biosynthesis (Walther et al., 2003). Also, the possibility that serotonin from plantain may have been transported across the blood brain barrier by the 5-HT transporter could be imagined, in line with the research by Nakatani and his team in 2008 which showed that augmented brain serotonin crosses the blood brain barrier through the 5-HT transporter.

In conclusion, this study shows that plantain diet increased brain levels of serotonin in mice. Further studies to elucidate the mechanism of the serotonergic potential of plantain should be encouraged.

## Acknowledgement

We thank Professor E.E. Osim, Dr. O.E. Ofem, Department of Physiology and Mr. J. Iwasam, Department of Medicine, University of Calabar, for their technical assistance during the study and statistical analysis. We also appreciate the management of Niger Delta University, for the encouragement to research

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