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SEROTONIN PATHWAY INVOLVEMENT IN SUPPRESSED PERCEPTION OF PAIN IN PLANTAIN DIET FED-MICE

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ABSTRACT

We investigated the involvement of serotonin receptors in the observed suppression of spontaneous perception of pain in mice that consumed unripe plantain diet for 30 consecutive days. Hot plate and formalin tests were used to study Aδ-fiber and C-fiber responses to pain stimulus in groups of mice that were fed plantain, plantain + ritanserin (serotonin antagonist) or normal rodent chow (control). Serotonin and 5-hydroxytryptamine concentrations in the brains of the mice were measured by High Performance Liquid Chromatography technique at the end of the pain response studies. The result shows that latency of jump was significantly ($p<0.001$) lower in the plantain + antagonist group compared to the plantain group. The frequency of hind paw lick and duration of paw attention were significantly ($p<0.001$) higher in the plantain + antagonist group than plantain group. The concentration of brain serotonin was significantly ($p<0.001$) higher in plantain group than control, while the concentration of 5-Hydroxytryptophan was significantly ($p<0.05$) lower in the plantain groups than control. Meanwhile, serotonin concentration was higher in the plantain + antagonist group than plantain group. These observations suggest that plantain diet suppressed the spontaneous perception of pain in mice through a mechanism that involves serotonergic pathways.

KEYWORDS: *Plantain, Pain, Serotonin, 5-Hydroxytryptophan, Ritanserin.*

INTRODUCTION

The processing of perception by supraspinal (brain) mechanism involving interactions of ascending and descending pathways is increasingly recognized to play major role in the representation and modulation of pain experience. Understanding those modulatory mechanisms in health and in disease is critical for developing fully effective therapies for the treatment of clinical pain conditions. Glutamate and substance P are neurotransmitters secreted by pain nerve endings and they mediate transmission of fast (A δ fiber) and slow (C- type fiber) pain impulses, respectively. But the analgesia system of the body in the brain that blocks synaptic transmission of pain, involves the neurotransmitter serotonin. (Sembulingam & Sembulingam, 2010).

Plantain contains serotonin (5-HT) and its precursor5-hydroxytryptophan (5-HTP) (Feldman & Lee, 1985). Since serotonin is known to mediate analgesia mechanism in the brain, it is conceivable that consumption of plantain diet may be beneficial as supplement in the management of clinical pain conditions. In view of these, we investigated the effect of consumption of plantain diet on pain perception in mice as well as the probable mechanism; to see if serotonin pathway is involved, by administering ritanserin which is an antagonist of serotonin receptors, 5-$HT_{2A}$and 5-HT_{2C}.

MATERIALS AND METHODS

Sixty Swiss mice (45-70 days, weighing between 23 – 38g) were separated into two set of 30 mice. Each set had 3 groups of 10 mice each. They were maintained in individually ventilated cages, 34cm by 19cm by 13cm under a normal light/dark cycle. The three groups in the first set were, control group (that was fed normal rodent chow), mixed diet or 50% plantain group (that had 50% plantain+ 50% rodent chow) and 100% plantain diet group (that was fed only plantain). The groups in the second set include control group (that had normal rodent chow), 100% plantain group (that had only plantain) and a third group designated plantain + antagonist group (that was fed 100% plantain alongside ritanserin administration). Ritanserin (0.03mg/kg) was administered intraperitonealy once daily before all the animals were fed, according to the method of Zomkowski*et al* (2004). All the animals had access to clean drinking water ad libitum. Animals were habituated at least for one week to the experimental room before experiment started. The feedings and administrations lasted for 30 days before the pain studies were done.

Preparation of Plantain Diet: Bunch of plantain was purchased from the central market in Calabar, Nigeria. The peel were removed and the pulp washed, chopped into slices and oven dried at 40^0C, 55% humidity for 24hrs. The dehydrated slices were then grinded into powder in a grinding mill, in line with the method of Babayemi *et al*(2010). This was the plantain diet. The mixed diet was prepared from plantain powder (flour) by measuring equal gram weights of rodent chow, plantain flour and mixing thoroughly.

TEST FOR PAIN PERCEPTION

Hot Plate Test: This is a test of thermal nociception, model of short duration stimuli. Each mouse was exposed to a hot surface whose temperature was maintained at 55±0.5˚C for maximum 30 seconds. The time it took for each mouse to start licking its foot pad was recorded and time taken for it to jump (Latency of jump) was recorded. These behaviours are the most common measures of pain threshold, and are considered supraspinally integrated (Le Bars et al., 2001).

Formalin Test: The formalin test for nociception involved injecting a noxious substance (3% formalin solution) into the plantar surface of the mice hind paws. The animal reacts to the formalin injection by licking and flinching the injected hind paw. The frequency and the duration of hind paw licks and attention were recorded over a period of one hour. The neurogenic response of formalin-induced behaviour reflects activation of C fiber primary afferent nociceptors (Ito *et al*., 2001). This test was in two phases. The response within the first 30 seconds following formalin injection reflects the perception of acute pain, while response during the later period shows chronic pain perception.

NEUROCHEMICAL ANALYSIS

The method of Mosienko et al (2012) was used. Animals were anaesthetized with ethyl chloride. Brains were removed, weighed and snap frozen on dry ice. The frozen tissue were homogenized on lyses buffer (containing 10µM ascorbic acid and 18% perchloric acid); centrifuged for 30 minutes at 20,000g, 4˚C and the supernatant was used for HPLC analysis. Sample separation occur at 20C˚on C 18 Reversed-phase column using a 10µM potassium phosphate buffer, pH 5.0, containing 5% methanol and at a flow rate of 2ml/min. Fluorescence of 5-Hydroxytryptophan (5-HTP) and serotonin (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.

RESULTS

In the hot plate test, the results of the comparison of the latency of jump in the first set of mice shows that the plantain fed-mice had significantly ($p<0.05$) higher jump latencies than control. In the formalin test to study acute and chronic pain responses, the frequency of hind paw licking and duration of paw attention were both reduced in the plantain fed-mice than control ($p<0.05$) in both acute and chronic phases.

However, when the serotonin receptors were blocked in the second set of experiment, these observations appear to reverse; The plantain + antagonist group of mice now expressed lower latency of jump, increased frequency of hind paw licking, increased duration of paw attention than the 100% plantain group($p<0.001$). Serotonin concentration in the brains of the mice which was increased in the 100% plantain fed-mice than control in the first set of experiment, was also significantly ($p<0.05$) increased in the plantain + antagonist group than 100% plantain group ($p<0.001$).

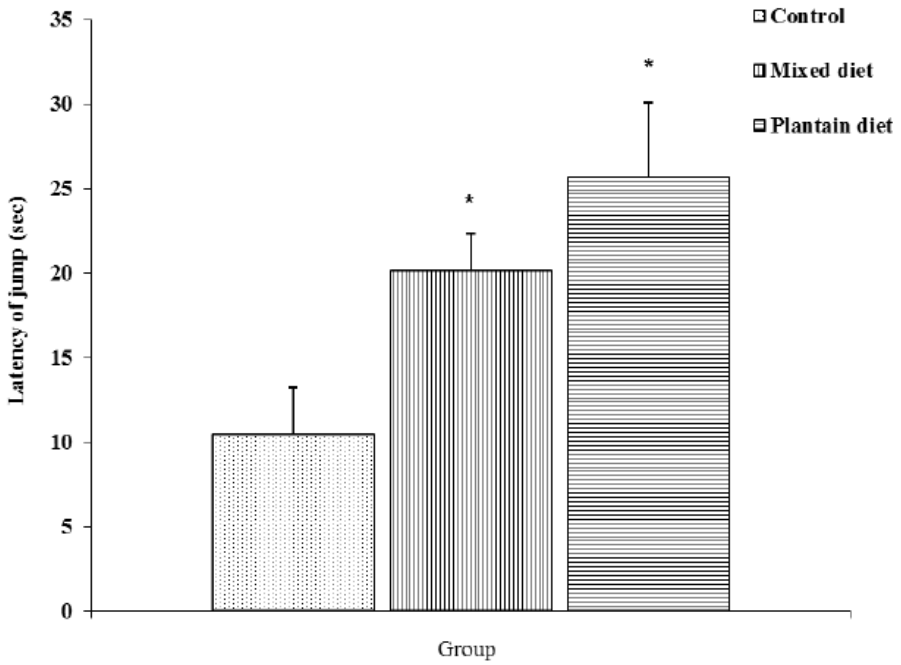

FIG 1: Comparison of latency of jump in the hot plate test in different experimental groups. Values are mean + SEM. *p<0.05 vs control

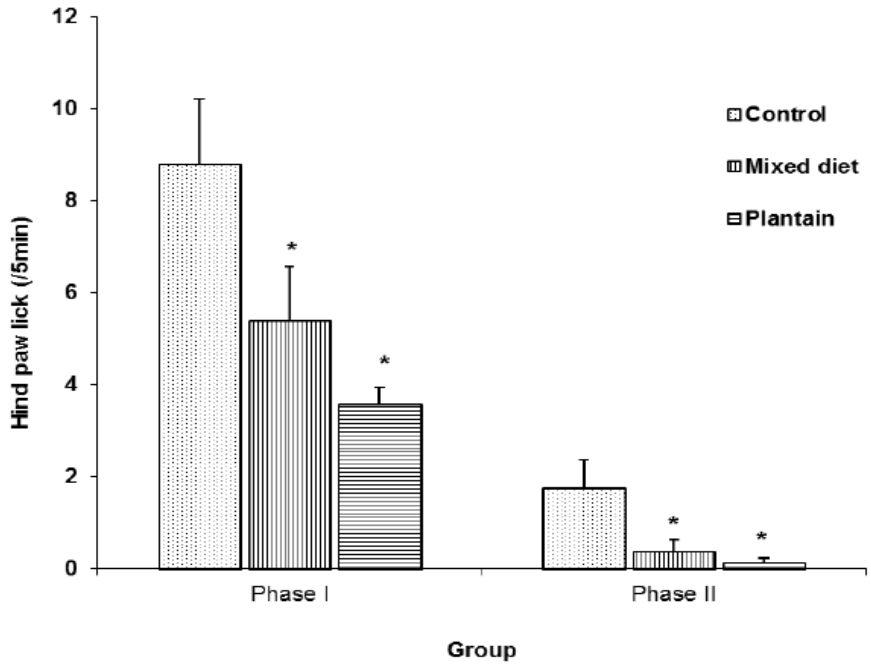

FIG 2: Comparison of frequency of hind paw licks in the formalin test in the different experimental groups. Values are mean + SEM, n = 9. *p<0.05 vs control

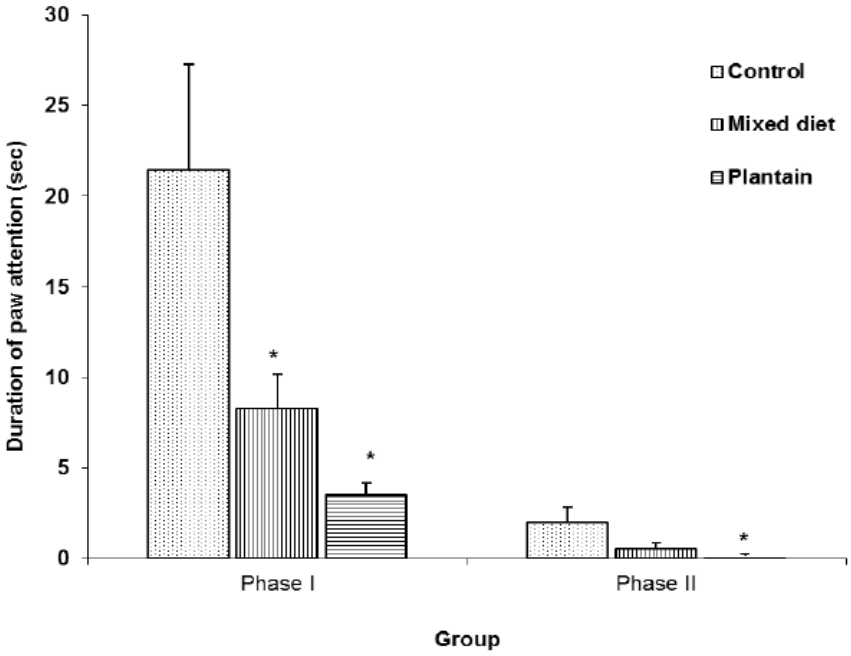

FIG. 3: Comparison of duration of paw attention in the formalin test in the different experimental groups. Values are mean + SEM, n = 9. *p<0.05 vs control ; a = p< 0.05 vs mixed diet

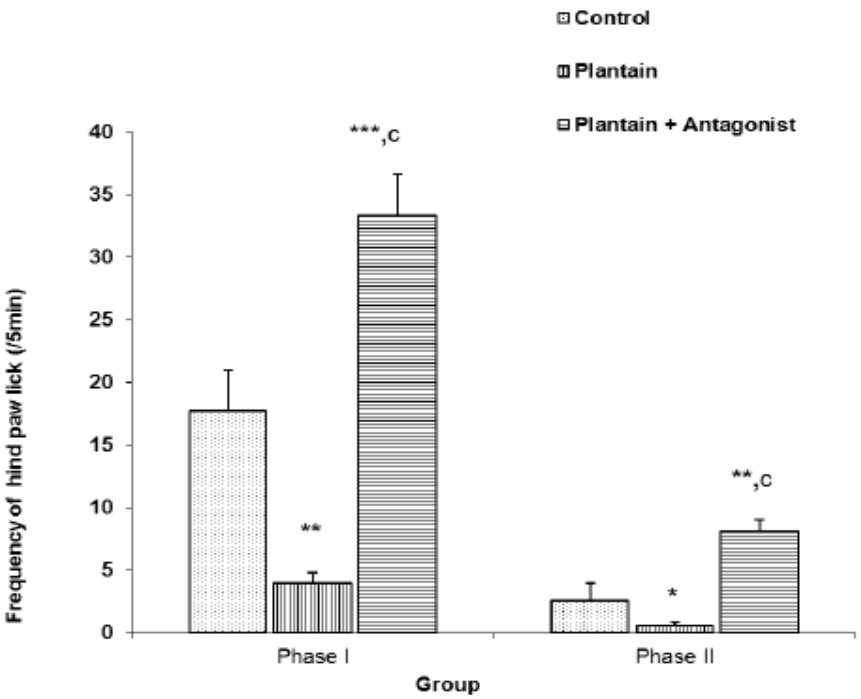

FIG. 4 Comparison of frequency of hind paw licks in the formalin test in the different experimental groups. Values are mean + SEM, n = 9. *p<0.05, **p<0.01, ***p<0.001 vs control; c = p<0.001 vs plantain.

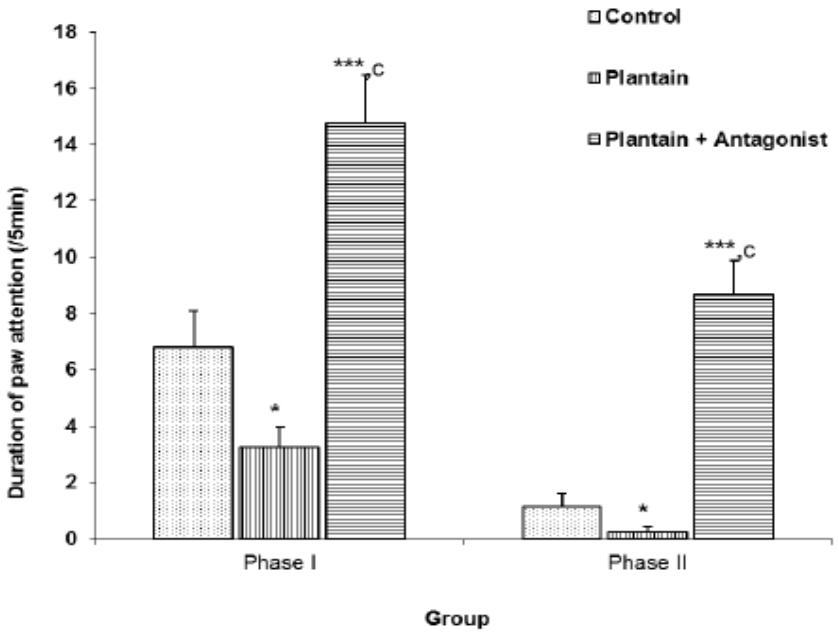

FIG. 5 Comparison of duration of paw attention in the formalin test in the different experimental groups. Values are mean + SEM, n = 9. *p<0.05, **p<0.01, ***p<0.001 vs control; c = p<0.001 vs plantain.

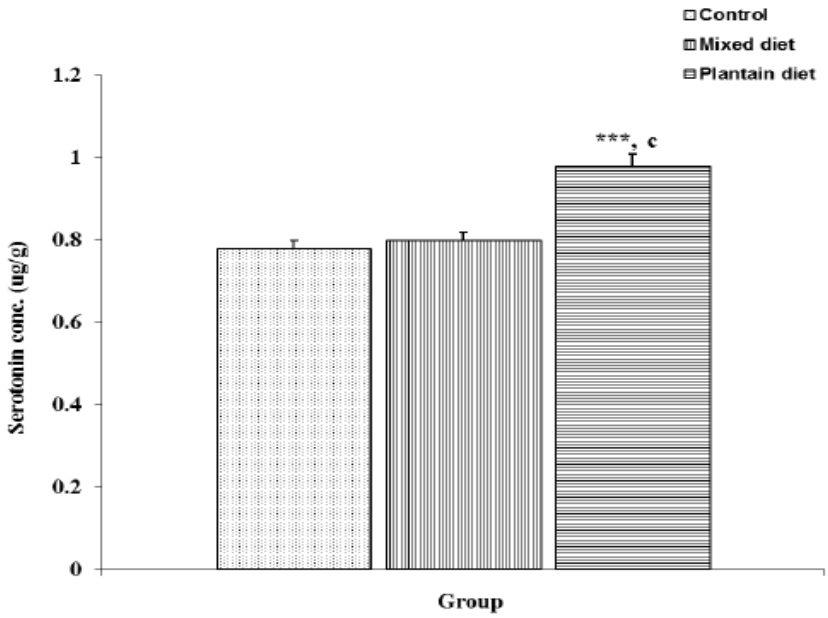

Fig. 6 Comparison of brain serotonin concentrations in the different experimental groups. Values are mean + SEM. ***p<0.001 vs control; c = p<0.001 vs mixed diet.

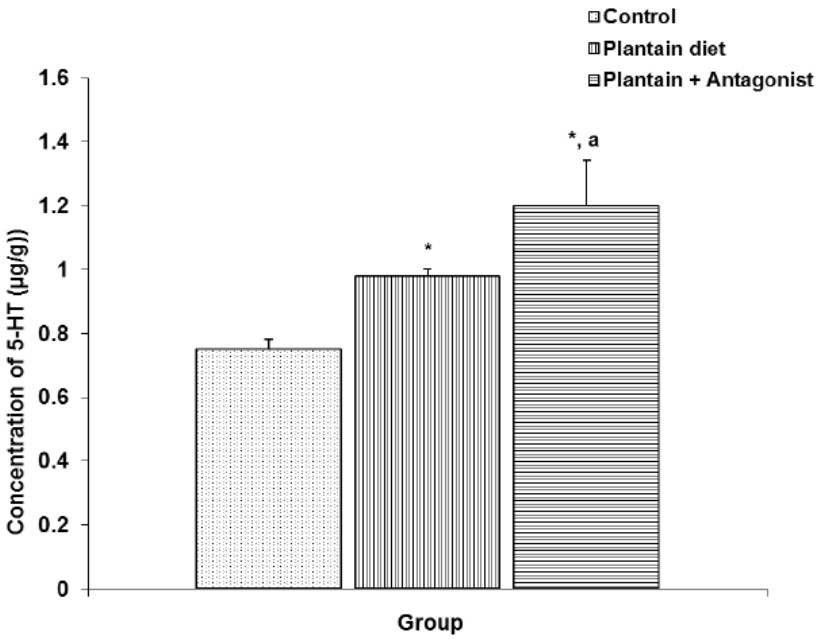

FIG. 7 Comparison of brain 5-HT (serotonin) concentrations in different experimental groups. Values are mean ± SEM. *p<0.05 vs control; a = p<0.05 vs plantain diet.

DISCUSSION

The hot plate test responses such as paw shaking, jumping and sometimes screaming is a complex pattern of willed, supra-spinal organized behaviour rather than a simple reflex. It is used to assess acute pain which involve A-δ fibers (Osim, 2008; Carter & Shieh, 2010).In the hot plate test, the latencies of jump by the 100% plantain and 50% plantain (mixed) diet groups of mice were significantly higher than that of control mice that consumed normal rodent chow. This indicates that it took longer time for plantain fed-mice to perceive pain than the control group of mice.

The formalin test model of pain study assesses both acute (short duration) pain and chronic (Long lasting or inflammatory) pain which usually involve C-type fibers. The frequency of hind paw lick for the 100% and 50% plantain groups were significantly lower in phases I (acute pain response) and II (chronic pain response), suggesting that acute and inflammatory pain perceptions are less in the mice that consumed plantain. The trend was similar for the duration of paw attention except that in phase II; the 50% plantain group was not significantly lower than control, but the 100% plantain group was significantly lower than control.

When serotonin receptors, 5-HT_{2A} and 5-HT_{2C} were blocked by ritanserin administration in the mice that consumed plantain, the responses to different pain perceptions were reversed during the hot plate test and formalin test. The latency of jump was reduced, the frequency and duration of hind paw lick as well as paw attention were increased in the plantain + antagonist group of mice when compared with 100% plantain group. These show that perception of pain was now increased in plantain fed mice when serotonin receptors were blocked.

Serotonin in brain is known to stimulate release of encephalin which blocks pain control gate in analgesia system. It also inhibits substance P which transmits chronic pain impulses and increases flexibility of veins, arteries and capillaries which makes them less likely to generate pain (Sembulingam & Sembulingam, 2010). Plantain contains serotonin as well as its precursor, 5-hyroxytryptophan that crosses the blood brain barrier. It is possible that plantain suppressed perception of pain because it increases serotonin in the brain, since the preliminary neurochemical analysis shows that serotonin level was significantly increased in the plantain groups than control while its immediate precursor (5-HTP) was decreased .However, in the plantain + ritanserin group of mice, the antagonist blocked 5-HT_{2A}and5-HT_{2C}receptors, thereby preventing the action of serotonin on its 5-$HT_{2A}$and 5-HT_{2C}receptors. This lack of action of serotonin on its receptors (as seen in the increased serotonin levels from the neurochemical analysis, figure 7) probably impaired the stimulatory effects of serotonin in the analgesia system, hence the increased perception of pain in the plantain + ritanserin group of mice.

CONCLUSION

The current investigation suggests that plantain diet may have suppressed the spontaneous perception of pain in mice through mechanism that involves serotonergic pathway.

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