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Neurobehavioural Effects of Pain Sensation in Mice following Chronic Consumption of Cooked Beans Diet (*Vigna unguiculata*)

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ABSTRACT

Pain may be defined as an unpleasant sensory and emotional experience, associated with actual or potential tissue damage. Therefore this study was designed to investigate the effect of chronic consumption of cooked beans (*Vigna unguiculata*) on pain sensation using three groups of Swiss mice (control and test) weighing 18g-30g (n=10 each).The control received normal rodent chow, while the test group received 50g of cooked beans in 50g of rodent chow per day and serotonin precursor (5HTP) (0.2mg/50g w/w) diet. Water was given *adlibitum* while daily food and water intake, as well as body weight changes, were monitored during the 31-day study. The tail flick and formalin tests were used to assess pain sensation. The results showed that the latency of tail flick was significantly higher in the test group compared to control ($p<0.05$).Thus showing decrease pain sensation. However, in the formalin test, the frequency and duration of paw attention in both phases of the test was significantly lower ($P< 0.05$) compared to the control group. The duration and frequency of paw licks ($P<0.05$) was also significantly different in the cooked beans diet and serotonin precursor group compared to the control. Therefore, chronic consumption of cooked beans diet may decrease pain sensation.

Keywords: Cooked beans, *Vigna unguiculata,* pain sensation, tail flick and mice.

INTRODUCTION

Bean offers a superb source of protein, carbohydrates, dietary fibre, minerals, vitamins and many phenolic compounds (1). Nowadays, researchers are particularly interested in the high antioxidant activities observed in beans. Bean is a very nutritious food from many aspects and it is not surprising that nutritionists would characterize beans as a nearly perfect food (2; 3).It is has been reported that beans have anticarcinogenic, anti-mutagenic (4) anti – inflammatory, anti-diabetic,

hypoglycaemic, depurative, cardio-protective and antioxidant effects (5). It has also been reported that beans contain serotonin and its precursor 5-Hydroxytrytophan (5-HTP) (6).Beans contain other chemical compounds including saponins, tannins, glycosides, flavonoids etc. Among the array of chemical constituents, notably, serotonin has neurobehavioural actions such as mood, memory, learning, and sleep (7).Serotonin has been shown to act (*Caenorhabditis elegans*) as neurotransmitter to modulate behaviour in response to changing cues, acting on both neurons and muscles to affect egg laying, pharyngeal pumping, locomotion and learning(8).Since beans contain neurotransmitters and chemicals that can potentially affect behavioural patterns, it may be worthwhile to find out whether long-term consumption of cooked and uncooked beans diet can affect behaviour. This was of particular interest when we consider the challenges that confront human behaviour and how behavioural disorders still remain a global concern (9).Human behaviour is believed to be influenced by endocrine and nervous system. 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 the human) 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 (10).

Unfortunately, many consumers avoid eating beans they fear that excessive flatulence may result (11). Thus, the challenge was to research into an affordable alternative of addressing the ever increasing incidence of learning, emotional or behavioural problems. A lot of the drugs in use for the management of behavioural disorders have many side effects such as blurred vision, dry mouth, drowsiness, muscle spasms or tremors and rapid weight gain, to mention a few(12).If foods that provide nourishment can contribute in the management of behavioural problems, it will be worthwhile to research into some of our local stable foods which may be beneficial in the management of behavioural disorders,

since foods have fewer side effects because than drugs and other therapies. Therefore, this research on cooked beans (*Vigna unguiculata*) is worthwhile for the public to know whether Nigeria beans will be beneficial or harmful in the listed neurobehavioral parameter, namely; pain sensation.

MATERIALS AND METHODS

Experimental animals/grouping: Thirty Swiss white mice of both sexes weighing between (18-30g) and bred at the animal room of the Department of Human Physiology, University of Nigeria, Nsukka, were used for this study after approval by the College Ethical Committee of Abia State University. The animals were transported to the animal house of Department of Physiology, Abia State University, Uturu, Nigeria, were they were acclimatized under standard laboratory conditions and given free access to normal feed and clean drinking tap water. The animals were randomly assigned into two groups, control and a test group. The animals in the control group received normal feed (rodent chow) only; while the test group received mixed feed of 50g powdered tobacco per every 50g of rodent chow (1% of tobacco diet) for 30 days. This is sequel to the fact that the determined LD50 for intraperitoneal administration of beans was 937.04mg/kg.

Experimental design: The tail flick and the formalin test were used to assess pain sensation in mice. The Tail flick nociceptive assay as developed by D'Amour and Smith (13) was used to assess the effect of powdered tobacco on pain. Water was boiled to a steam at 100°c, and a portion of this hot water was taken and some cold water was added to reduce the temperature of the hot water to 49°c in which the experiments were carried out. This temperature was constantly maintained by adding cold water when the temperature is above 49°c or by adding hot water when the temperature decreases below 49°c before each experiment was carried out. Thermometer was also constantly immersed into the hot water to ensure accurate temperature is maintained.

The mouse was restrained loosely, gently handled and care was taken to prevent bite. A stop watch was set and started exactly at the time when the tail of the mouse was immersed into the hot water at 49°c. The stop watch was stopped exactly when the mouse flicked its tails from the hot water. This time was recorded as the latency of tail flick. The experiment was repeated after 1 hour for each mouse. The experiments were carried out on both the control and the test groups, after the experiment, the animals were returned to their metabolic cages thereafter. The experiment lasted for a day.

Also, the formalin test has been regarded as being a more satisfactory model of clinical pain than the hot plate tests (14). Mice were carried into the room in their home cages. Each mouse was picked at the base of its tail and 0.2ml of 2.5% formalin was injected into the right hind paw of the mouse using a needle and syringe. The animal was placed in the observation box and observed for five (5) minutes. The animal was then returned to its cages and allowed for thirty (30) minutes before it was taken back to the observation box to be re-observed for another five (5) minutes. This procedure was repeated for each animal. The experiment lasted for a day and the behaviours' scored were; (1) frequency of paw lick and paw attention, (2) frequency and duration of grooming and (3) frequency of stretch attend posture.

Statistical Analysis: Data between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Post-hoc using Newman-Keuls. Data were presented as Mean ± SEM and a "P" value less than 0.05, was considered statistically significant.

RESULTS

Frequency of right hind paw attention following formalin administration

The frequency of attention to the right hind paw injected formalin in the early phase of the test of the cooked beans and serotonin precursor fed mice was significantly lower compared to control ($P<0.05$). The

frequency values were 24.00 ± 2.07/5 min for control; 11.43 ± 1.39/5 min and 6.00±0.82 respectively in the first trial after 5 minutes of formalin administration for cooked beans and serotonin precursor diet fed mice (Figure 1). However, the serotonin precursor fed mice was also significantly lower ($p<0.05$) compared to cooked beans group of mice. The frequency of attention to the right hind paw of the cooked beans and serotonin precursor fed mice in the late phase was also significantly lower ($P<0.05$) compared to control. The values were 1.20 ± 0.47/5 min (control); 0.57 ± 0.37/5 min and 0.43±0.30/5min for cooked beans and serotonin precursor diet fed group (Figure 1).

Duration of right hind paw attention following administration of formalin

The duration of times the animals (mice) in both groups' pay attention to their hind paw of which formalin was injected were 89.38 ± 11.33secs for the control group, 58.64 ± 8.90secs and 39.03±5.51 seconds for the mice fed cooked beans and serotonin precursor diet in the early phase of the test and 2.60 ± 0.60secs (control) and 0.92 ± 0.71seconds (cooked beans fed diet) and 0.55±0.39 seconds (serotonin precursor fed diet) in the late phase. The duration of right hind paw attention of the cooked beans and serotonin precursor fed mice was significantly lower ($p<0.05$) compared to the control in both phases (Figure 2).

Duration of right hind paw lick following formalin administration

The duration of paw lick to the right hind paw injected formalin in the early phase of the test of the cooked beans and serotonin precursor fed mice was significantly different compared to control ($P<0.05$). However, the serotonin precursor fed mice was also significantly lower ($p<0.05$) compared to cooked beans group of mice. The duration values were 26.79 ± 2.56secs for control; 17.75 ± 4.43secs and 8.67±2.08 seconds respectively in the first 5 minutes for cooked beans and serotonin precursor diet fed mice (Figure 3).The duration of paw lick to the right hind paw of the cooked beans and serotonin precursor fed mice in the late

phase was also significantly different ($P<0.05$) compared to control. The values were 13.30 ± 0.52secs (control); 7.96 ± 0.51secs and 4.16±0.16 seconds respectively for cooked beans and serotonin precursor diet fed group (Figure 3).

Frequency of right hind paw lick following administration of formalin

The number of times the animals (mice) in both groups' licks their hind paw of which formalin was injected were 14.90 ± 1.59/ 5min for the control group; 8.86 ± 1.68/5 min and 5.71±0.42 respectively in the first trial after 5 minutes of formalin administration for the mice fed cooked beans and serotonin precursor diet in the early phase of the test and 6.87 ± 0.22/5 min (control); 2.57 ± 0.30/5 min (cooked beans fed diet) and 0.14±0.14/5min(serotonin precursor fed diet) in the late phase. The frequency of right hind paw lick of the cooked beans and serotonin precursor fed mice was significantly shorter ($p<0.05$) compared to the control in both phases (Figure 4).However, frequency of paw lick in the serotonin precursor diet fed group was shorter than in the cooked beans group of mice ($p<0.05$).

Latency tail flick

The latency of tail flick, which indicate the time it takes for the mice to flick its tail from the warm water, showed that the values for control; cooked beans and serotonin precursor diet fed mice were 10.32 ± 3.66 sec; 15.45 ± 2.86 and 18.99±3.16 seconds respectively (tail 1) and 8.30± 2.07; 12.40± 2.82secs and 24.31±1.96 seconds (trail 2). The latency of flicks by the cooked beans and serotonin precursor fed mice was significantly higher ($p<0.05$) compared to that of the control (Figure 5). However, the serotonin precursor fed mice was significantly higher compared to cooked beans group of mice.

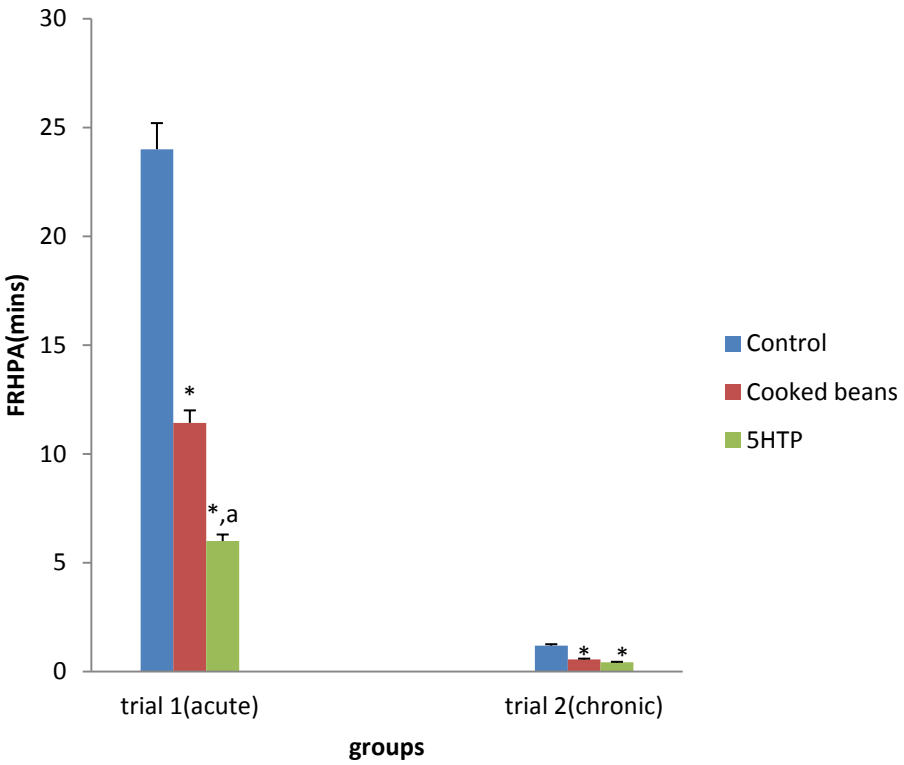

Fig 1: frequency of right hind paw attention of the different experimental groups after two trials during the formalin test for assessment of pains. . Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control;a = p<0.05 vs. cooked beans.

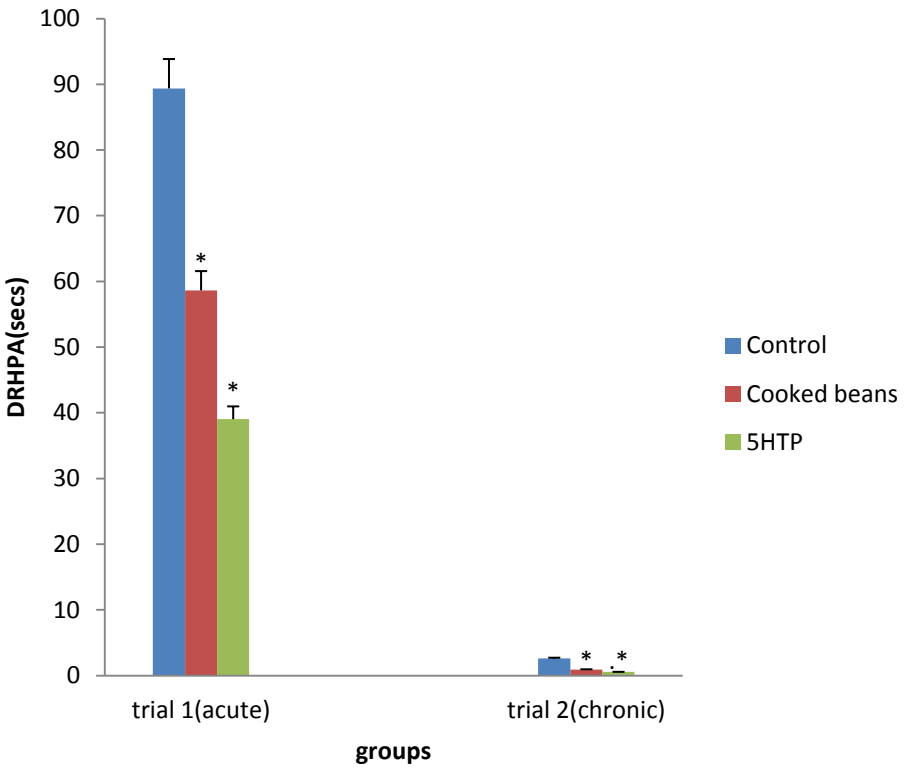

Fig 2: Right hind paw duration of the different experimental groups after two trials during the formalin test for pain assessment of pains. . Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control.

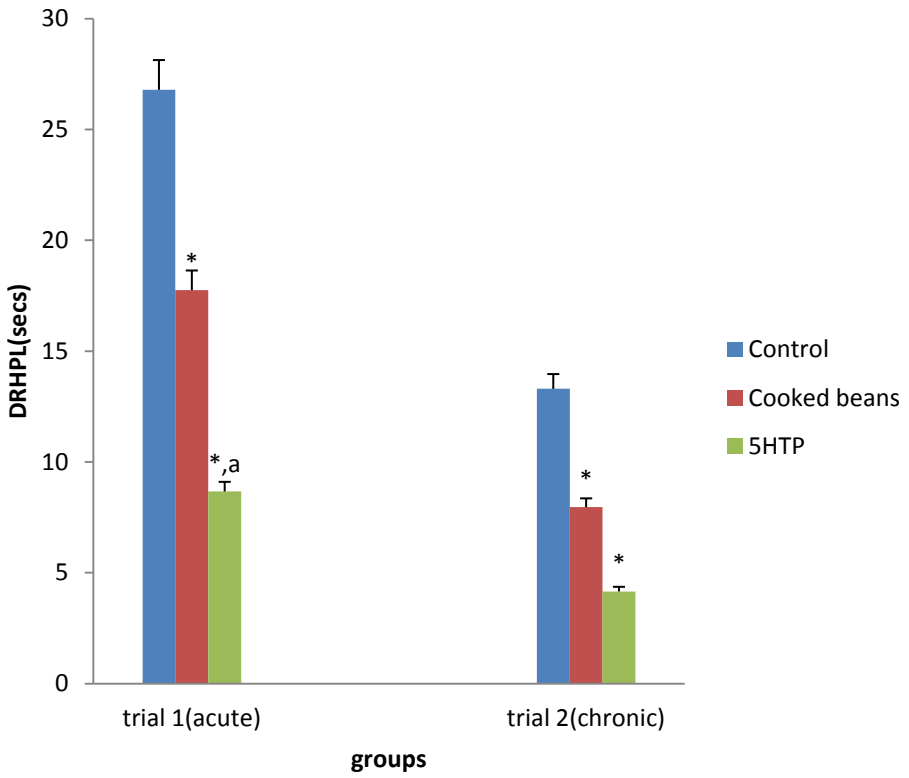

Fig 3: Right hind paw lick duration of the different experimental groups after two trials during the formalin test assessment for pains. . Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control;a = p<0.05 vs. cooked beans.

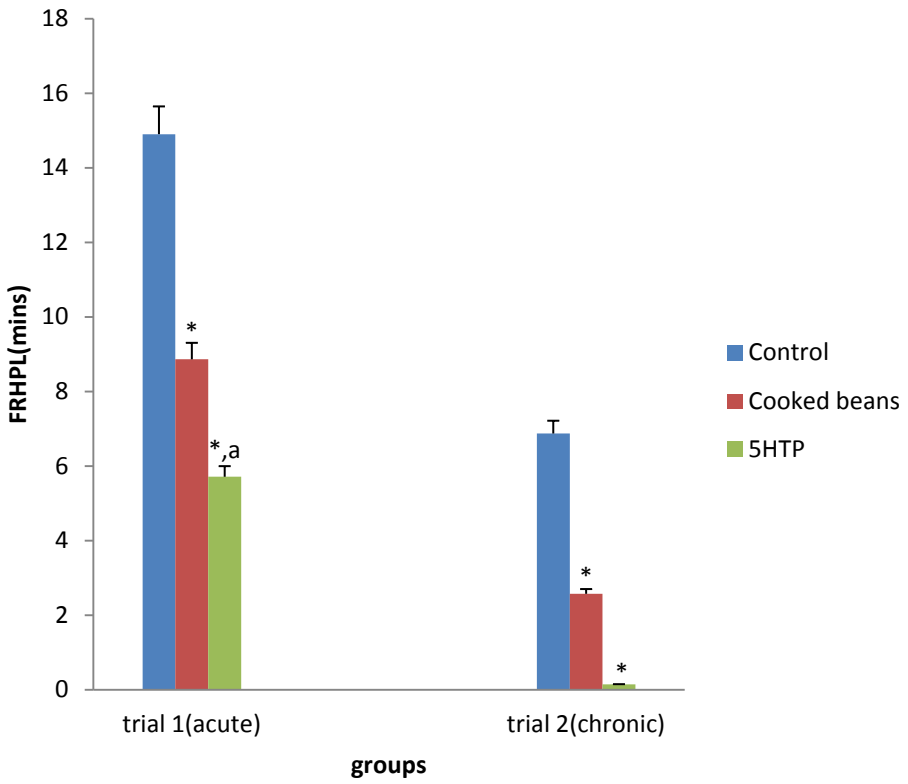

Fig 4: frequency of right hind paw lick of the different experimental groups after two trials during the formalin test for assessment of pains. Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control;a = p<0.05 vs. cooked beans.

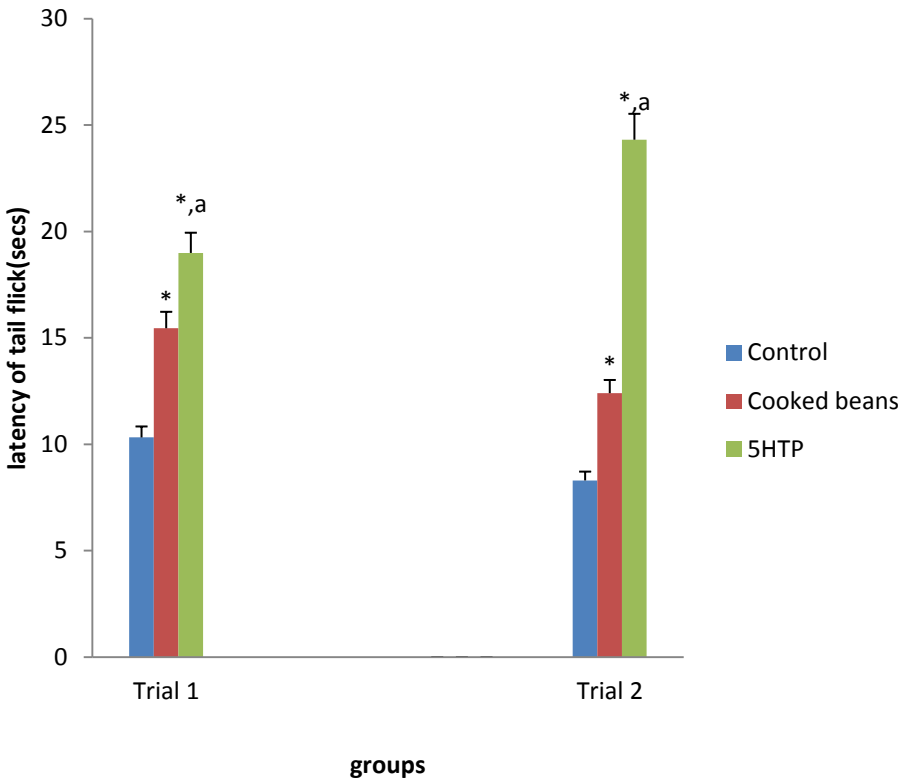

Fig 5: latency of tail flicks of the different experimental groups. . Values are expressed as are expressed as mean ± SEM, n = 10,*p<0.05 vs. control;a = p<0.05 vs. cooked beans.

DISCUSSION

In this study, the animal models of physiological pain assessment used include, tail flick test, formalin test (15).Tail flick is a system feature that involves when radiant heat is applied on the animal's tail-when the animal feels discomfort, there is a sudden tail movement (tail flick).This has proved that it is particularly a sensational property of pharmacological substance. It can also be used to evaluate basal thermal pain sensitivity or the study of putative genetic differences among animals without drugs. (16).In the experiment, the tail flick test showed that the latency of tail flick for the group of mice fed cooked beans and serotonin precursor diet were significantly higher than the control. This indicates that the pain threshold for the test mice was raised when

compared to the control, implying that mice took longer time before they perceived pain. These longer latencies indicate a raised pain threshold and thus decreased in pain perception following the consumption of cooked beans diet. This result means that beans affect the spontaneous response to the sensation of pain.

The neurogenic response of formalin-induced behaviour reflects activation of C fibre primary afferent nociceptors (15).This test was in two phases. The response within the first 30 seconds following formalin injection is the perception of acute pain, while the later period shows chronic pain perception. Frequency of hind paw attention and hind paw-licking following injection with formalin was defined as the number of times the mice lick or shake their hind paw after injection with formalin. Lower frequencies of hind paw attention and hind paw licking indicate analgesic effect while higher frequencies indicate hyperalgesia.

The result showed that during acute and chronic phases of pain, the beans diet- fed mice and that of the serotonin precursor fed mice had significantly less pain perception compared to control, since the frequencies and durations of hind paw lick and hind paw attention following formalin injection was significantly lower in the beans and serotonin precursor diet- fed mice than the control. The report of Roefofs and Perkins (17) showed a relationship between fear and pain-that decrease in fear would decrease pain and vice versa. The result is also consistent with decrease in fear related behaviour such as stretch attends posture and grooming and decrease in pain sensation.

Pain reduction was observed on the first and second phases of pain following chronic consumption of beans diet. The first phase was the fast or pricking pain mediated by the type A-delta fibres that release the neurotransmitter glutamate while the second phase represent slow pain where inflammation of tissues occurs. Slow or chronic pain is mediated by the neurotransmitter; substance P (18).It is therefore interesting to

note that beans diet can be beneficial in the reduction of chronic pain if the results in mice can be extrapolated to man.

The Serotonin circuitry is a well-established pathway involved in brain's analgesia system during transmission of pain in the central nervous system. It is known that the analgesic fibres of this system release neurotransmitters that inhibit pain transmission to the brain, and the neurotransmitters released by the fibres of analgesic pathway are serotonin and enkephalins(18; 19).

This result suggests that the mice fed with cooked beans and serotonin precursor diet were less sensitive to pain, when compared to those fed with the control diet. The decrease in the sensitivity to pain caused by the beans diet may also be attributed to the presence of flavonoids and phlorotannins in the beans which has been reported to reduce pain perception due to their anti-inflammatory properties (20).In conclusion, our findings suggest that cooked beans diet reduces pain sensation in mice. This may be so because beans contain 5-HTP (serotonin precursor) and 5-HT (serotonin) that plays a positive role in the brain analgesia system. A second set of experiments implicated the serotonergic pathway, as the threshold for pain perception was increased in the mice that consumed the serotonin precursor diet.

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