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Research Article

THE AMELIORATING EFFECT OF UNCOOKED BEANS DIET IN CD-1 MICE

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ABSTRACT

Beans contain serotonin and its precursor, 5-hydroxytryptophan (5-HTP), which have neurobehavioral actions on memory, anxiety, mood, and pain. This study was, therefore, designed to investigate the ameliorating effect of uncooked beans on pain sensation using three groups of Swiss white mice (control and test) weighing 18 g–35 g (n=10 each). The control group received normal rodent chow, while the test group received 50 g of uncooked beans in 50 g of rodent chow per day and serotonin precursor (5-HTP) (0.2 mg/50 g w/w) diet. Water was given *ad libitum* while daily food and water intake, as well as body weight changes, were monitored during the 30-day study. The formalin tests were used to assess pain sensation. The results showed that 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 ($p<0.05$) were also significantly lower in the uncooked beans diet and serotonin precursor group compared to the control. Therefore, consumption of uncooked beans diet may decrease pain sensation.

Keywords: Beans, Pain sensation, Formalin and mice.

INTRODUCTION

Pain can simply be referred as an emotional feeling which is associated with tissue damage. It is, therefore, a protective mechanism in the body. Beans are considered as good source of protein content, complex carbohydrates, dietary fibers, and some minerals and vitamins [2]. In addition to these nutritional components, common beans are rich in a variety of several phytochemicals with potential health benefits such as polyphenolic compounds, flavonoids, saponins, alkaloids, glycosides, tannin, lectin, trypsin inhibitor, and phytic acids, among others [3,6]. It has also been reported that beans contain serotonin and its precursor 5-hydroxytryptophan (5-HTP) [10]. Since beans contain neurotransmitters and chemicals that can potentially affect behavioral patterns, it may be worthwhile to find out whether long-term consumption of uncooked beans diet can affect behavior, such as pain sensation.

MATERIALS AND METHODS

Experimental animals/grouping

A total of 30 Swiss white mice weighing between 18 and 35 g and bred at the animal room at the Department of Human Physiology, University of Abia State University. The animals 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 50 g powdered uncooked beans per every 50 g of rodent chow (50% of the uncooked beans diet) and (0.2 mg/50 g) serotonin precursor diet for 30 days.

Experimental design

The formalin test was used to test for pain sensation as developed by Abbott et al. [1]. Mice were carried into the room in their home cages. Each mouse was picked by the base of its tail and 0.2 ml 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 5 min. The animal was then returned to its cages and allowed for 30 min before it was taken back to the observation box to be reobserved for another 5 min. This procedure was repeated for each animal.

Behavior scored during the pain test included the following:

- Frequency of the right hind lick/scratch
- Frequency of the right hind paw attention
- Duration of attention.

Statistical analysis

Data obtained from the experiments were statistically analyzed using Microsoft Excel, with factorial ANOVA/t-test in the statistics program start view version for Windows or Mac. Post hoc comparison was also done using the student ± Newman-Keuls design. Values were represented as mean ± standard error of the mean and $p<0.05$ was considered as statistically significant.

RESULTS

Fig. 1 shows the frequency of the right hind paw licks for mice fed normal, uncooked beans, and serotonin precursor diets as 14.90±1.59, 7.71±0.75, and 5.71±0.42/5 min, respectively, in the first trial, after 5 min of formalin administration denoting acute pain. The frequency of paw lick for the mice fed uncooked beans and serotonin precursor diet was significantly shorter ($p<0.05$) than that of the control fed mice. In the second trial, after 30 min of formalin administration denoting chronic pain, the frequency of paw licks was 0.87±0.22, 0.14±0.14, and 0.14±0.14/5 min for mice fed normal, uncooked beans, and serotonin precursor diet, respectively. The frequency of the right hind paw lick in the uncooked beans and serotonin precursor diet-fed mice was significantly shorter ($p<0.05$) than those of the control fed mice.

The values for the duration of hind paw lick following administration of normal, uncooked beans, and serotonin precursor diets were 26.79±2.56, 17.75±2.32, and 8.67±2.08 s, respectively, in the first 5 min. The duration of hind paw lick in the uncooked beans and serotonin precursor fed mice was significantly lower ($p<0.05$) compared to control. In the second trial, after 30 min of formalin administration, the hind paw lick duration in the group of mice fed normal, uncooked beans, and serotonin precursor diets was 1.30±0.52, 0.16±0.16, and 0.16±0.16 s, respectively. The duration of hind paw lick was significantly lower in the uncooked bean and serotonin precursor fed mice compared to the control group ($p<0.05$) (Fig. 2).

The values for the frequency of hind paw attention following administration of normal, uncooked beans, and serotonin precursor diet were 24.00±2.07, 8.14±1.18, and 6.00±0.82/5 min, respectively, in the first trial, after 5 min of formalin administration. The frequency of hind paw attention was significantly lower in the uncooked beans and serotonin precursor fed mice compared to control ($p<0.05$). In the second trial, after 30 min of formalin administration, the values were 1.20±0.47, 0.43±0.30, and 0.43±0.30. The frequency of hind paw attention was significantly lower in the serotonin precursor and uncooked beans group compared to control ($p<0.05$) (Fig. 3).

The values for the duration of hind paw attention following administration of normal, uncooked beans, and serotonin precursor diet were 89.38±11.33, 53.59±4.14, and 39.03±5.51 s, respectively. The duration of hind paw attention fed with uncooked beans and serotonin precursor was statistically shorter than those fed with control diet ($p<0.05$). In the second trial, after 30 min of administration of formalin, the duration of paw attention was 2.60±0.60, 0.37±0.24, and 0.55±0.39 s, respectively. The duration of hind paw attention was significantly lower in the uncooked beans and serotonin precursor fed mice compared to control ($p<0.05$) (Fig. 4).

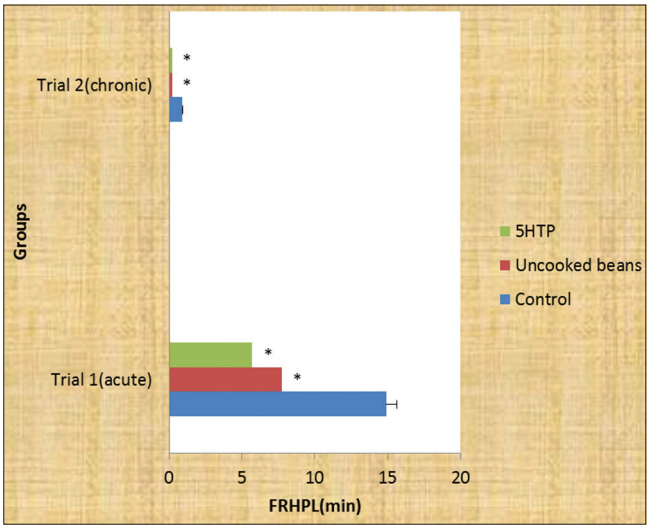

Fig. 1: Right hind paw lick frequency of the different experimental groups after two trials during the assessment of pain using formalin. Values are expressed as mean ± standard error of the mean, n=10,*p<0.05 versus control

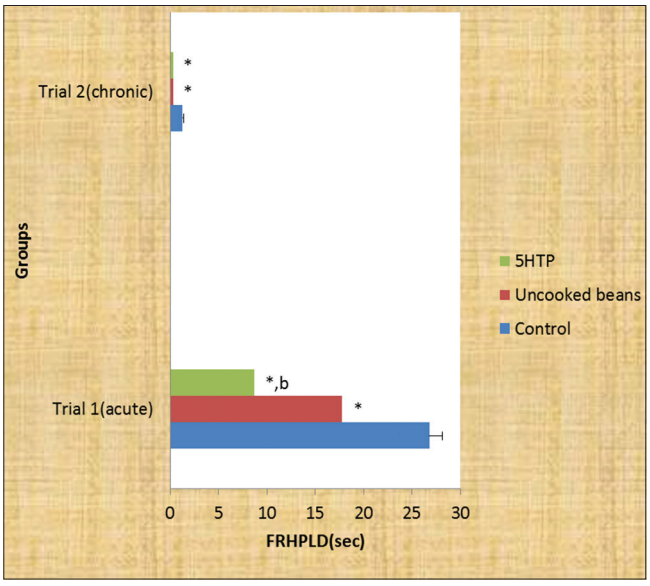

Fig. 2: Right hind paw lick duration of the different experimental groups after two trials during the formalin test assessment for pains. Values are expressed as mean ± standard error of the mean, n=10,*p<0.05 versus control; b = p<0.05 versus uncooked beans

DISCUSSION

The response of formalin-induced behavior reflects activation of C fiber primary afferent nociceptors [5]. This test was in two phases. The response within the first 30 s 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

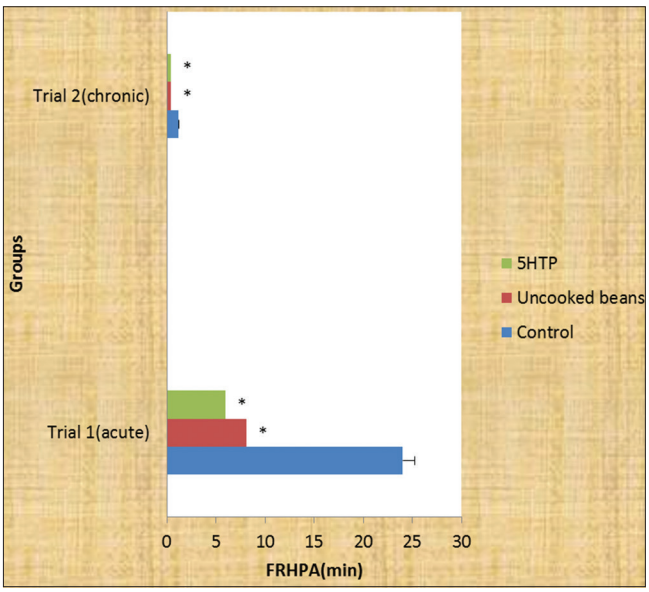

Fig. 3: Frequency of the right hind paw attention of the different experimental groups after two trials during the formalin test assessment for pains. Values are expressed as mean ± standard error of the mean, n=10, *p<0.05 versus control

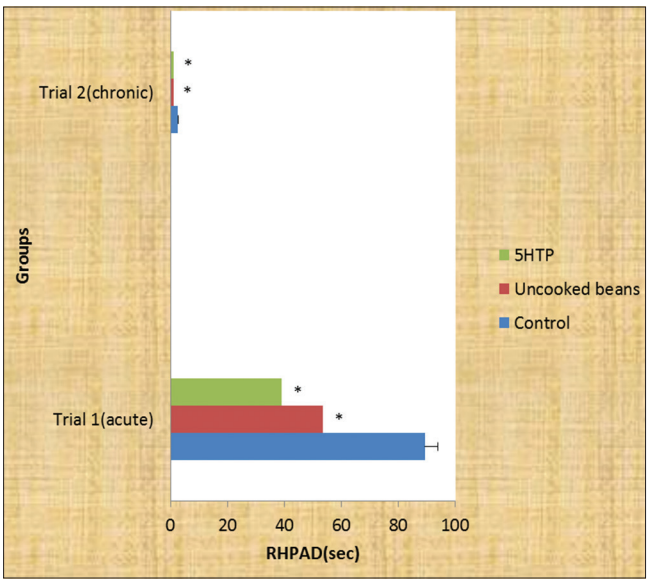

Fig. 4: Right hind paw attention duration of the different experimental groups after two trials during the formalin test assessment for pains. Values are expressed as mean ± standard error of the mean, n=10, *p<0.05 versus control

following injection with formalin was defined as the number of times the mice licks or shakes 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. Our finding 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 were significantly lower in the beans and serotonin precursor diet-fed mice than the control. Pain reduction was observed in the first and second phases of pain following chronic consumption of beans diet. 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 fibers of this system release neurotransmitter that inhibits pain transmission to the brain, and the neurotransmitters released by the fibers of analgesic pathway are serotonin and enkephalins [8,12]. Our findings suggest that uncooked beans and serotonin precursor diet mice showed less sensitive to pain when compared to those fed with the control diet. Beans diet may decrease pain sensitivity which may also be due to the presence of flavonoids and phlobatannins in the beans which has been reported to reduce pain perception due to their anti-inflammatory properties [4,7]. Finally, uncooked beans diet reduces pain sensation in mice. This may be so because beans contain 5-HTP (serotonin precursor) and 5-HT (serotonin) that play a positive role in the brain analgesia system. The 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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AUTHORS' CONTRIBUTIONS

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