Effects of Diets Containing Black-eyed Beans and Serotonin Precursors on Neuromuscular Coordination in Experimental Mice

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Abstract

Background and Aim: Black-eyed beans contain the neurotransmitter serotonin and its precursor, 5-hydroxytryptophan (5-HTP) which is known to have neurobehavioral effects on neuromuscular coordination. Therefore, the aim of this study was to find whether long-term consumption uncooked bean diet has effects on some neurobehavioral parameters notably; neuromuscular coordination using Swiss white mice as experimental animals. **Methods:** Thirty CD1 mice were randomly assigned into three groups, namely, control, uncooked beans diet (50% w/w), and serotonin precursor (5-HTP) diet (0.2 mg/50 g w/w) for 30 days. All the mice had access to clean drinking water *ad libitum*. Before the neurobehavioral parameters were assessed, the LD_{50} of the beans and that of the serotonin precursor (5-HTP) was determined. Serotonin concentration was measured in beans using gas chromatography analysis. Neuromuscular coordination was investigated alongside food and water intake and body weight change. The involvement of serotonin pathway was investigated using the set of mice administered serotonin precursor for comparison with the beans diet-fed mice. Beam walking was used to assess neuromuscular coordination. **Results:** The results showed that beans and 5-HTP-fed mice showed better neuromuscular coordination when compared to control. This is because, decreased frequency of foot slips and longer latency of falls indicates a higher level of maneuverability in the beam, thus indicating better neuromuscular coordination. **Conclusion:** Long-term consumption of uncooked beans improves neuromuscular coordination.

Keywords: 5-hydroxytryptophan, beam walk and mice, beans

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Introduction

There are many varieties of dry bean classes depending on the color, shape, and size. Some of the commonly consumed varieties are navy, black, kidney, and pinto beans. Bean is used as staple food in Nigeria and globally[1] and are a superb source of protein, carbohydrates, dietary fiber, minerals, vitamins, and many phenolic compounds.[2] Bean is a very nutritious food[3,4] and has been reported to exhibit that beans have anticarcinogenic, antimutagenic[5] anti-inflammatory, antidiabetic, hypoglycemic, depurative, cardioprotective, and antioxidant effects.[6] Beans are rich in serotonin and its precursor 5-hydroxytryptophan (5-HTP).[7] They also contain saponins, tannins, glycosides, and flavonoids.[7] A key feature of serotonins is the regulation of neurobehavior such as mood, memory, learning, and sleep.[8] Evidence suggest that serotonin acting as a neurotransmitter on both neurons and muscles can modulate behavior in response to changing cues, to affect egg laying, pharyngeal pumping, locomotion in the roundworm *Caenorhabditis elegans*.[9] Since black-eyed beans contain serotonin and 5-HTP and chemicals that can potentially affect behavioral patterns, it may be worthwhile to investigate whether long-term consumption of uncooked black-eyed beans diet can affect behavior. This is of particular interest when considering the challenges that confront human behavior and how behavioral disorders still remain a global health concern.[10] Therefore, this study explores the effect

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of long-term consumption of uncooked black-eyed beans on behavior such as neuromuscular coordination.

Materials and Methods

Experimental animals/grouping

Thirty adult Swiss white mice weighing between 15 and 30 g obtained from the disease-free stock of the animal house, Department of Physiology, University of Nigeria, Nsukka, were used for this research work. Animals were kept under standard laboratory conditions in well-ventilated rooms, with a temperature of about 25°C ± 4°C, 12 h of daylight, and 12 h of darkness. The animal care was according to the principles of laboratory animal care (National Institutes of Health) and national laws of animal care. Appropriate approval was also obtained from the Local Ethical Committees. The animals were randomly assigned into three groups of ten animals per group. The animals in the control group (1) received normal rodent feed (rodent chow) only while the test group received mixed feed of 50 g of uncooked beans per every 50 g of rodent chow making 50% of the beans diet (test Group 2) and (0.2 mg/50 g) serotonin precursor diet (Group 3) for 30 days.

Experimental design

The beam has a length of 100 cm, a width of 2 cm and is elevated to a height of 40 cm. The beam is marked at 5 and 1 cm intervals. It is composed of wood and is coated with black paint. The camera is located 175 cm above the beam. The subject is scored live and filmed with a video camera.

The mice were carried to the test room in their home cages. The mouse was removed from its home cage and placed at one end of the balance beam. After the mouse has secured its grip on the beam, the trial begins. The maximum length of the trial is five minutes. The mouse was tested under white light, during the dark phase. The beam is cleaned with 70% ethanol and permitted to dry between each trial.

The following parameters were measured: distance traveled (the number of line crossed), foot slips (number of times one of the mouse's back feet slips from the beam), number of turns (frequency that the animal reversed direction), and latency to fall (time at which the animal fell off of the beam). If a fall occurred, the animal was not placed back on the beam but was returned to the home cage.

Statistical analysis

Data between the groups were analyzed by one-way analysis of variance followed by *post hoc* using Newman–Keuls. Data were presented as mean ± standard error of mean, and $P < 0.05$ was considered statistically significant.

Results

The frequency of foot slips in different experimental groups was recorded as 7.20± 0.88; 4.38 ± 1.12 /5min and 2.71 ± 0.42/5min for mice fed with control, uncooked beans, and serotonin precursor diets, respectively. The frequency of foot slips for the uncooked beans was significantly lower than control group ($P < 0.05$) [Figure 1]. However, the serotonin precursor-fed mice was much significantly lower ($P < 0.01$) when compared with both control group and mice fed with cooked beans.

Figure 2 shows the distance covered in the different experimental groups which are recorded as 41.71 ± 3.99; 69.25 ± 7.29/5 min; and 85.50 ± 8.46 for mice fed control, cooked beans, and serotonin precursor diet, respectively. The distance covered for mice fed with cooked beans diet during beam walking was significantly higher ($P < 0.05$) compared to control. However, the serotonin precursor-fed mice was much significantly higher ($P < 0.01$) compared to both the control group and mice fed with cooked beans.

The latency of fall in different experimental groups is as follows: 7.36 ± 10.56; 13.9 ± 2.59; and 15.37 ± 3.48 s for control group, mice fed with uncooked beans, and serotonin precursor diet, respectively. The latency of fall was longer for the uncooked beans and serotonin precursor diet-fed mice compared with that of the control group $P < 0.05$ [Figure 3].

The number of turns in different experimental groups is as follows: 5.40 ± 0.46; 3.88 ± 0.46; and 6.00 ± 0.46/5 min for control group of mice, mice fed with uncooked beans, and serotonin precursor diet, respectively. There was no significant difference in the number of turns in both uncooked beans and serotonin precursor diet-fed mice when compared with that of the control group [Figure 4].

Discussion

The performance of animals (mice) in the beam walking apparatus was used as a measure of neuromuscular coordination, fatigue, and motor learning.[11] This performance also serves to monitor changes in sensorimotor coordination and balance and as a measure of ataxia. It has been used to study the effects of drugs on motor or neuromuscular coordination,

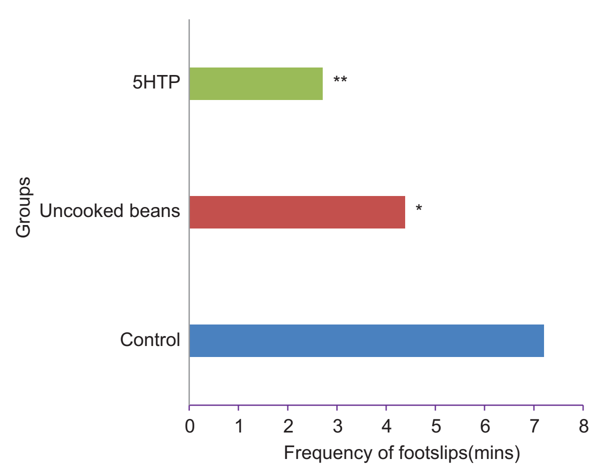

Figure 1: Frequency of foot slips for assessing motor coordination among the different experimental groups. Values are expressed as mean ± standard error of the mean, $n = 10$; *significantly different from control at $P < 0.05$; **$P < 0.01$ versus control

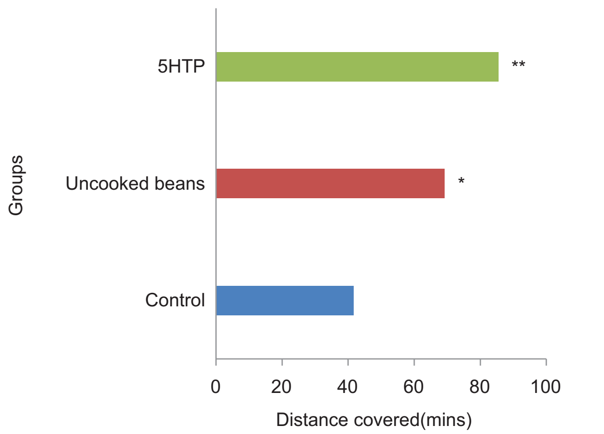

Figure 2: Distance covered for assessing motor coordination among the different experimental groups. Values are expressed as mean ± standard error of the mean, $n = 10$; *significantly different from control at $P < 0.05$; **$P < 0.01$versus control

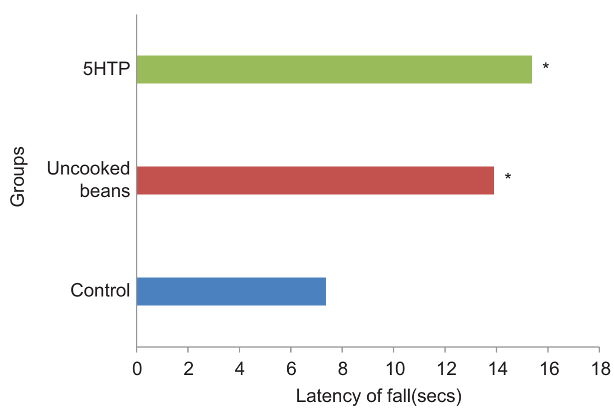

Figure 3: Latency of fall for assessing neuromuscular coordination among the different experimental groups. Values expressed as mean ± standard error of the mean, *significantly different from control at $P < 0.05$ versus control

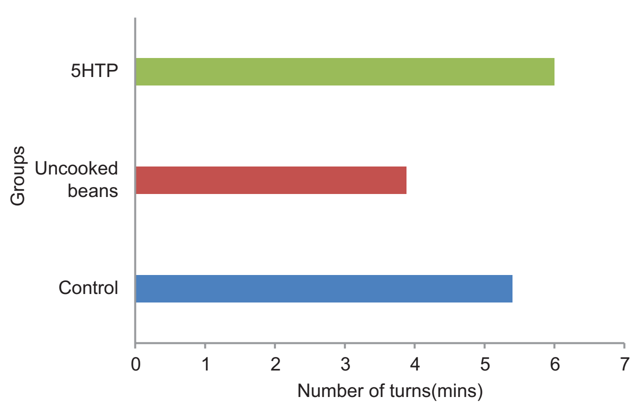

Figure 4: Number of turns for assessing neuromuscular coordination among the different experimental groups. Values expressed as mean ± standard error of the mean

cerebellar function, and motor learning.[12] The task involved in sensorimotor learning, where the mice learned to maneuver their way and stay on the beam balance for as long as possible.

The latency for the mice to fall decreased over trials and over days as the animal becomes more proficient in maneuvering on the apparatus and could stay on longer. The longer the latency of fall, the better the mice maneuverability, the better the motor (neuromuscular) coordination, and therefore, the better the motor learning ability. The results in the beam walking showed that the beans group showed better motor coordination compared to control. This is because, decreased frequency of foot slips and longer latency of falls indicates a higher level of maneuverability in the beam, thus indicating better motor coordination. This result is consistent with earlier work carried by.[13]

Since the cerebellum is involved in learned adjustments that make motor coordination easier when a given task is performed over and over,[14] consumption of beans may be enhancing cerebellar function. However, as the result of beans-fed mice was similar to serotonin-fed mice, the serotonin may probably be the chemicals in beans. Therefore, it can be concluded that the effects could be due to serotonin. However, this is a preliminary study to assess the effect of diets containing black-eyed beans rich in serotonin precursors on neuromuscular coordination in experimental mice models. We believe this study provides a strong hypothesis to explore the possibility of assessing diets rich in serotonin precursors on neuromuscular functions in human beings.

Limitations of the study

One of the major limitations of this study was that the levels of serotonin and its precursor were not estimated in beans-fed mice.

Conclusion

Consumption of beans diet improves neuromuscular coordination in mice. One of the active principles involved in these actions that are attributed to beans may be serotonin.

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Conflicts of interest

There are no conflicts of interest.

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