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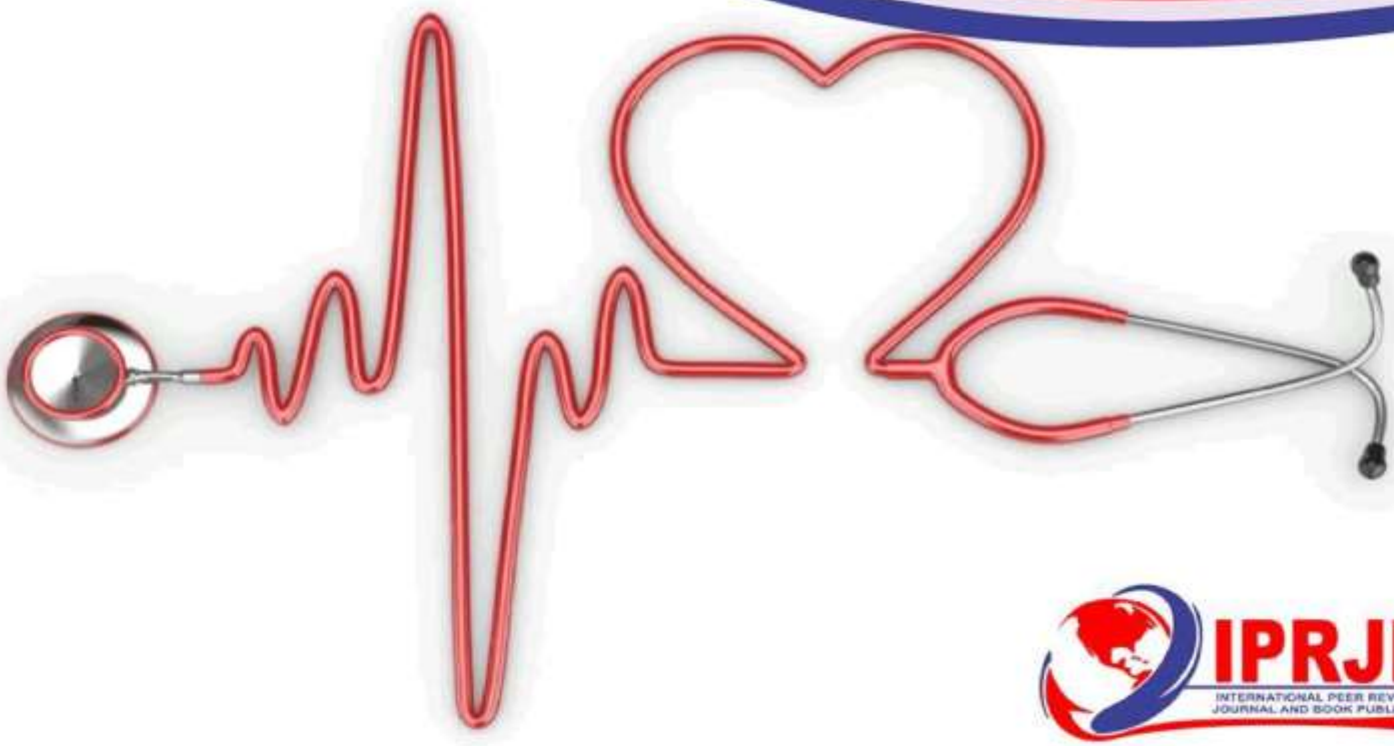

THE COMPARATIVE EFFECTS OF LOCOMOTOR/EXPLORATORY BEHAVIOUR FOLLOWING CHRONIC CONSUMPTION OF BEANS DIET (Vigna unguiculata) IN MICE

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ABSTRACT

Purpose: The effects of chronic consumption of beans (Vigna unguiculata) diet commonly called iron beans on locomotor/exploratory behaviour were investigated. Forty adult Swiss white mice (18-30g body weight), were used for the study.

Method: The open field maze and light and dark transition box were employed for the evaluation of locomotor/exploratory behaviour. Forty (40) CD1 mice were randomly assigned into four groups, viz; control, cooked beans diet (50% w/w), uncooked beans diet (50% w/w), while another set of mice were placed on serotonin precursor (5-HTP) diet (0.2mg/50g w/w) for thirty days. All animals were allowed free access to clean drinking water. Daily food intake, water intake and body weight change were measured. Before the neurobehavioural parameters were assessed, the phytochemical analysis of the beans, LD_{50} of the beans and that of the serotonin precursor (5-HTP) was determined. Serotonin and serotonin precursor (5-HTP) concentration were measured in beans using High performance liquid chromatography (HPLC) analysis.

Results: The frequency of rearing in the open field was not significantly different in the beans and serotonin precursor fed group compared to control. However, the frequency of line crosses, stretch attend posture and walling activity were decreased in the test group ($p<0.05$) compared to control. This indicates a decreased locomotor/exploratory behavior in the test group. There was also a significant ($p<0.05$) decrease in the frequency of transition in the light/dark transition box for beans and serotonin precursor fed group when compared to the control.

Conclusion: In conclusion, chronic consumption of beans diet decreases locomotor and exploratory behaviour.

Keywords: *Beans, locomotion/exploration, open field and mice*.

1.0 INTRODUCTION

Black eyed bean, a legume, is a subspecies of the cowpea, grown around the world for its medium-sized, edible bean. The bean mutates easily, giving rise to a number of varieties (Joseph and Holloway, 2010). The common commercial one is called California black eye; it is palecolour with a prominent black spot. The current accepted botanical name is *vigna unguiculata* subsp. *Unguiculata*, although previously it was classified in the genus *Phaseolus*.

The black-eye peas are actually a small, almost white bean with a black spot along their side originally brought to the United States by slave traders, this beans has been a popular food in the Southern US for hundreds of years. These beans, sometimes referred to as cowpeas, Southern pea, China pea, Cowgram in United State or Niebe in French speaking Africa (Henshaw *et al*., 2008; Sigh and Rachie, 1997; Kay, 1979). Cowpea is well known for its good source of dietary protein for human consumption and for animal feed in the tropics, especially in Africa, Brazil and India. It thrives well in hotter more arid climates and more infertile soils than other food legume crops due to its symbolic nitrogen fixing abilities which helps in maintaining soil fertility in peasant cropping systems. Over 65% of the cowpea crop is produced in Africa; Nigeria and Niger producing 50% of the World supply. The United States is the only developed country producing large amount of cowpea (Henshaw,2008). The main centres of cultivation of cowpea in Nigeria are Kano, Katsina, Bauchi, Bornu, Sokoto and Niger State in the North and Ibadan, Owo and Benin in the West (Oyenuga, 1968). Cowpeas, are also popular in Africa in different fermented dishes. In India, they are often eaten like lentils. Thin skinned black-eyed peas cooked up in only 30to 60 minutes and require no pre-soaking. Traditionally served with rice or corn bread, they remain a popular Southern Cuisine. 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 (Brunton *et al*., 2005).Serotonin has been shown to act (*Ceanorhabditis elegans*) as neurotransmitter to modulate behaviour in response to changing cues, acting on both neurons and muscles to affect egg laying and locomotion(Daniel & Micheal, 2007).Since beans contain neurotransmitters and chemicals that can potentially affect behavioural patterns, it may be worthwhile to find out whether long-term consumption of uncooked beans diet can affect behaviour such as locomotion. This was of particular interest when we consider the challenges that confront human behaviour and how behavioural disorders still remain a global concern (Messman, 2005).

Beans constitute a major portion of the Nigerian local diet. It contains neurotransmitters, notably serotonin that has neurobehavioural actions as well as its precursor, 5-Hydroxytryptophan that also has similar actions. It is conceivable therefore that long term consumption of beans diet can affect locomotor/exploratory behaviour.

2.0 MATERIALS AND METHODS

2.1 Experimental animals/grouping:

Forty (40) adult Swiss white mice weighing between 15-30g obtained from the disease -free stock of the animal house, Department of Physiology, University of Nigeria, Nsukka were used for this research work. The animals were randomly assigned into four (4) groups of ten (10) animals per group. Each mouse in a study group was individually housed in a plastic cage with iron gauze bottom grid and a wire screen top. The animal room was adequately ventilated, and kept at room temperature and humidity of 22±3^{0}c and 40-70% respectively with 12 hour natural light-dark cycle.

The animals in the control group received normal rodent feed (rodent chow) only, while the test group received mixed feed of 50g of cooked and uncooked beans per every 50g of rodent chow making 50% of the beans diet and(0.2mg/50g) serotonin precursor diet for 4 weeks. This is sequel to the fact that the determined LD50 for the intra-peritoneal administration of beans and serotonin precursor was 939.04mg/kg and 155.30mg/kg.

2.2 Experimental Design

The open field maze and light/dark transition box was used to access locomotor/exploratory behavior. The open field test was used to provide measures of locomotion, exploration and anxiety (Walsh and Cummins, 1976).The experiment was performed in an enclosed laboratory to screen the animals from noise and provide dim light to avoid distraction of the animals. The animals were placed in the centre of the maze and allowed to explore the open field for 5 minutes. Before introducing each animal; the floor of the maze was cleaned using 70% ethyl alcohol in order to eliminate olfactory influences. The following behaviours were scored during the 5 minutes to assess locomotor and exploratory behaviours: line crossing, rearing, stretch attend posture, walling and freezing.

Also, the light and dark transition box is a test of locomotion and exploratory behaviour. Each mouse was picked up using a plastic bucket and placed in the centre division of the large compartment facing the floor. The mouse was allowed to explore the transition box for 5 minutes. Entering into the chamber is defined as the placement of all four paws into the chamber. During the period of 5 minutes, behaviour scored using a stop watch was frequency of transition.

2.3 Statistical Analysis

Data collected were expressed a Mean SEM (standard error of mean), analysis of variance (ANOVA) and the student‘t’ test were used for analysis. “P” value less than 0.05, was considered statistically significant.

3.0 RESULTS

Open field maze:

Figure 1.0 compares the frequency of line crosses in the two groups of mice. The horizontal locomotor behaviour following consumption of uncooked beans and control diet was measured by the number of lines crossed by the animals (mice) within five minutes in the open field maze. The number of lines crossed by the mice were, 73.43± 3.60 (control); 68.70 ± 0.90/5min (cooked beans), 64.88 ± 0.89 (uncooked beans) and 53.43 2.00(serotonin precursor).

The statistical analysis shown in the graph of figure 1, shows that the frequency of line crosses of the cooked and uncooked beans was significantly different from control at ($p<0.05$).The serotonin precursor fed mice was significantly lower ($P<0.01$) compared to control. However, the frequency of line crosses of the serotonin precursor fed mice was significantly lower ($P<0.01$) compared to the cooked and uncooked beans group.

The frequency of rearing in the open field for the control group was 2.33 ± 0.94/5mins.This was not significantly different when compared to the cooked, uncooked beans and serotonin precursor group of mice which was 4.13± 1.17/5min, 1.88± 0.88/5min session and 12.57 0.48/5mins (serotonin precursor) respectively (Figure 2).

The frequency of walling in the group of mice fed cooked, uncooked beans and serotonin precursor diet was 19.00 ± 2.93/5min, 12.88± 2.79/5min session and 12.57± 2.38(serotonin precursor) in 5 minutes..). The frequency of walling in the group of mice fed cooked was significantly different from control at p<0.05.However, the uncooked beans and serotonin precursor diet fed mice were significantly different at (P<0.01) compared to control (Fig. 3).

Figure 4 compares the freezing frequency between the four experimental groups of mice respectively. The freezing frequency shown in figure 4 were, 0.90± 0.10(control); 1.75±0.20/5min (cooked), 2.00±0.30 (uncooked) beans /5mins and 2.43±0.20/5mins (serotonin precursor). The frequency of freezing among the group of mice fed cooked, uncooked beans and serotonin precursor diet was significantly higher (p<0.001) compared to control. However, the group of mice fed with the uncooked beans was significantly higher when compared to those fed with the cooked beans, whereas the serotonin precursor group was statistically higher when compared to those of the cooked and uncooked beans group.

The freezing duration shown in figure 5 were, 3.16± 1.51(control); 10.26±3.00 (cooked), 20.59±4.00seconds (uncooked) beans and 32± 1.41/5mins (serotonin precursor).The freezing duration in the group of mice fed cooked, uncooked beans and serotonin precursor was significantly higher (p<0.05) compared to control. However, the freezing duration in the group of mice fed uncooked beans was statistically higher when compared to the cooked beans group, whereas those fed with serotonin precursor diet were seen to be significantly higher(p<0.05) than those of the cooked and uncooked beans group.

Figure 6 compares the frequencies of the stretch attend posture (SAP) which is a measure of anxiety and exploration in the four experimental groups. The values are: 3.90± 0.12(control); 3.13±0.13/5min (cooked), 3.50±0.10/5mins (uncooked) beans and 2.86± 0.12(serotonin precursor). The frequency of stretch attend posture of the cooked, uncooked beans and serotonin precursor group was significantly lower (p<0.05) compared to control. However, the stretch attend posture in the group of mice fed uncooked beans was statistically higher when compared to the cooked beans group, whereas those fed with the serotonin precursor diet was significantly lower compared to the cooked and uncooked beans group.

The frequency of transition (that is the number of time the animals passes into the opposite compartment) between the two experimental groups is in (figure 7). The values are: 16.70±1.46 (control); 12.50 ± 2.46/5min (cooked), 12.50 ± 2.46/5mins (uncooked) beans and 5.43±0.65 (serotonin precursor).The frequency of transition of the frequency of transition of the uncooked beans group of mice was significantly lower (p<0.01) compared to control. However, the serotonin precursor fed mice was significantly lower (p<0.001) compared to control and at p<0.01 compared to cooked beans group.

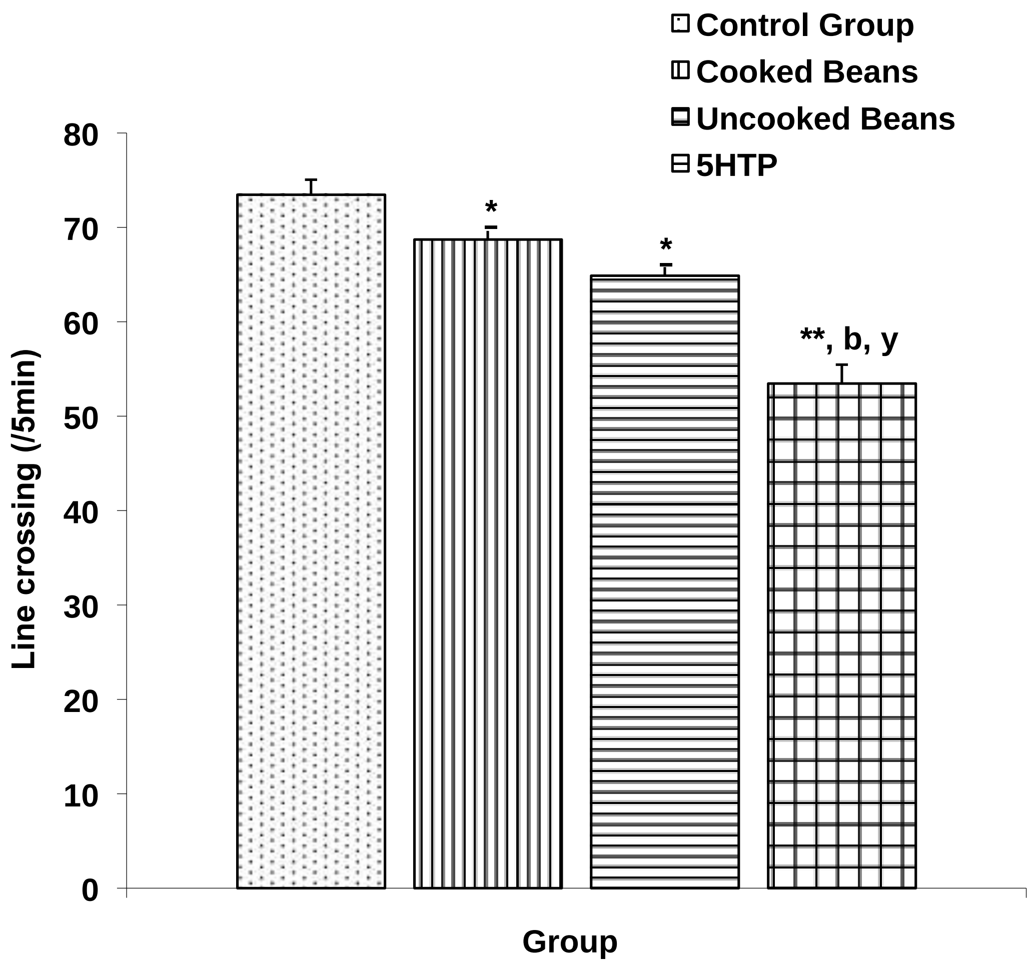

Figure 1: Frequency of line crossings in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
****p<0.01 vs control; b = p<0.01 vs cooked bean; y = p<0.01 vs uncooked bean.**

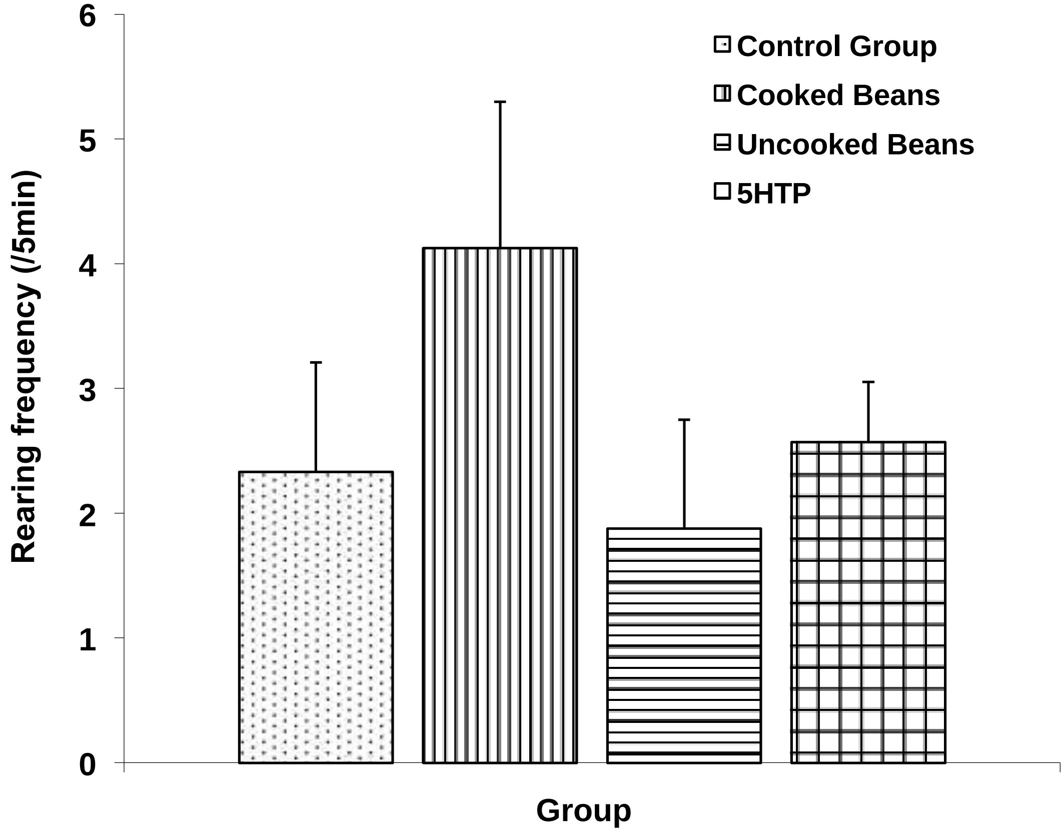

Figure 2: Rearing frequency in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
No significant differences among groups

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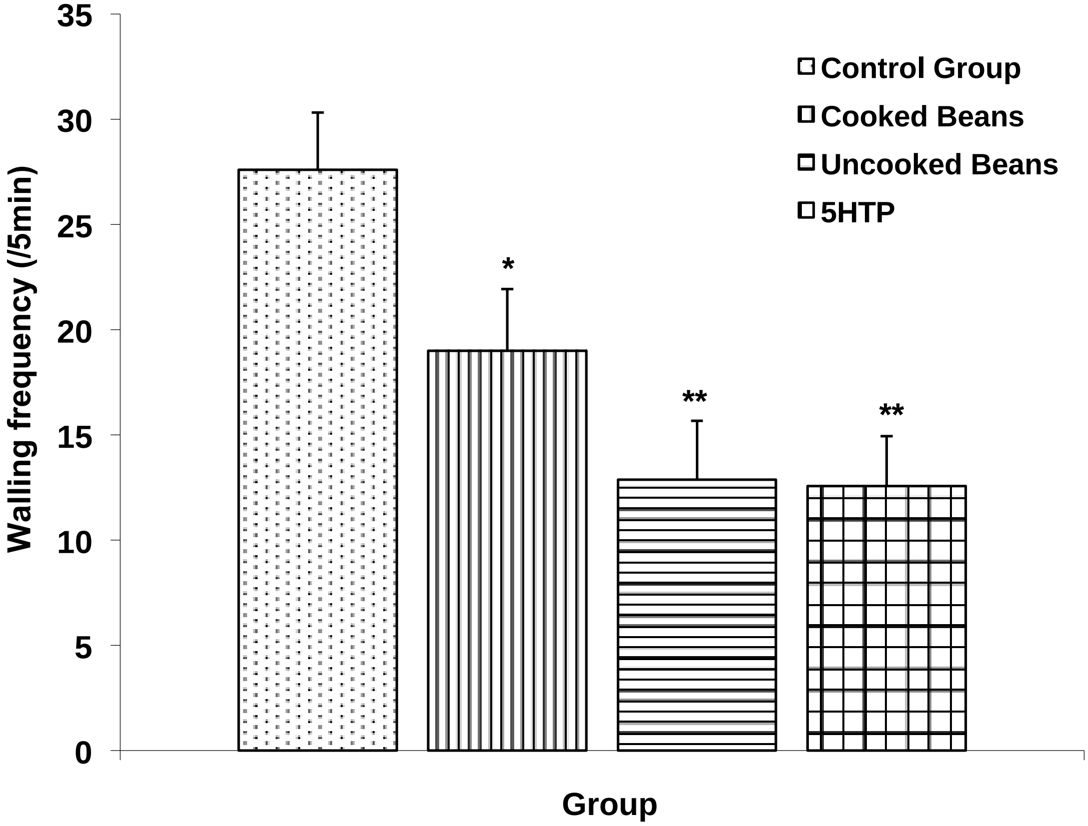

Figure 3: Walling frequencies in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
****p<0.01 vs control**

Control Group
Cooked Beans
Uncooked Beans

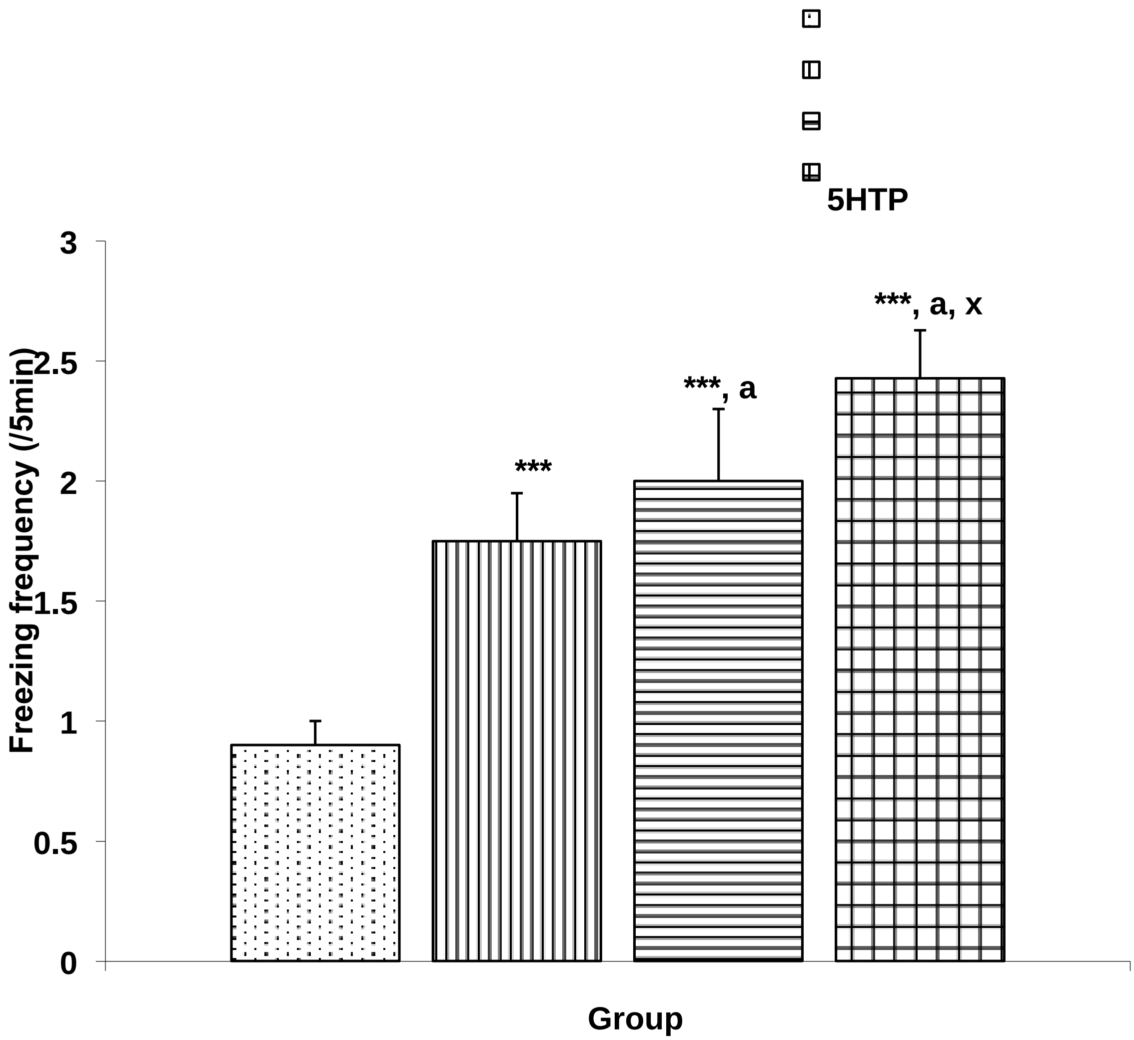

Figure 4: Freezing frequency in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
*****$p<0.001$ vs control; a = $p<0.05$ vs cooked bean; x = $p<0.05$ vs uncooked bean.**

Control Group
Cooked Beans
Uncooked Beans

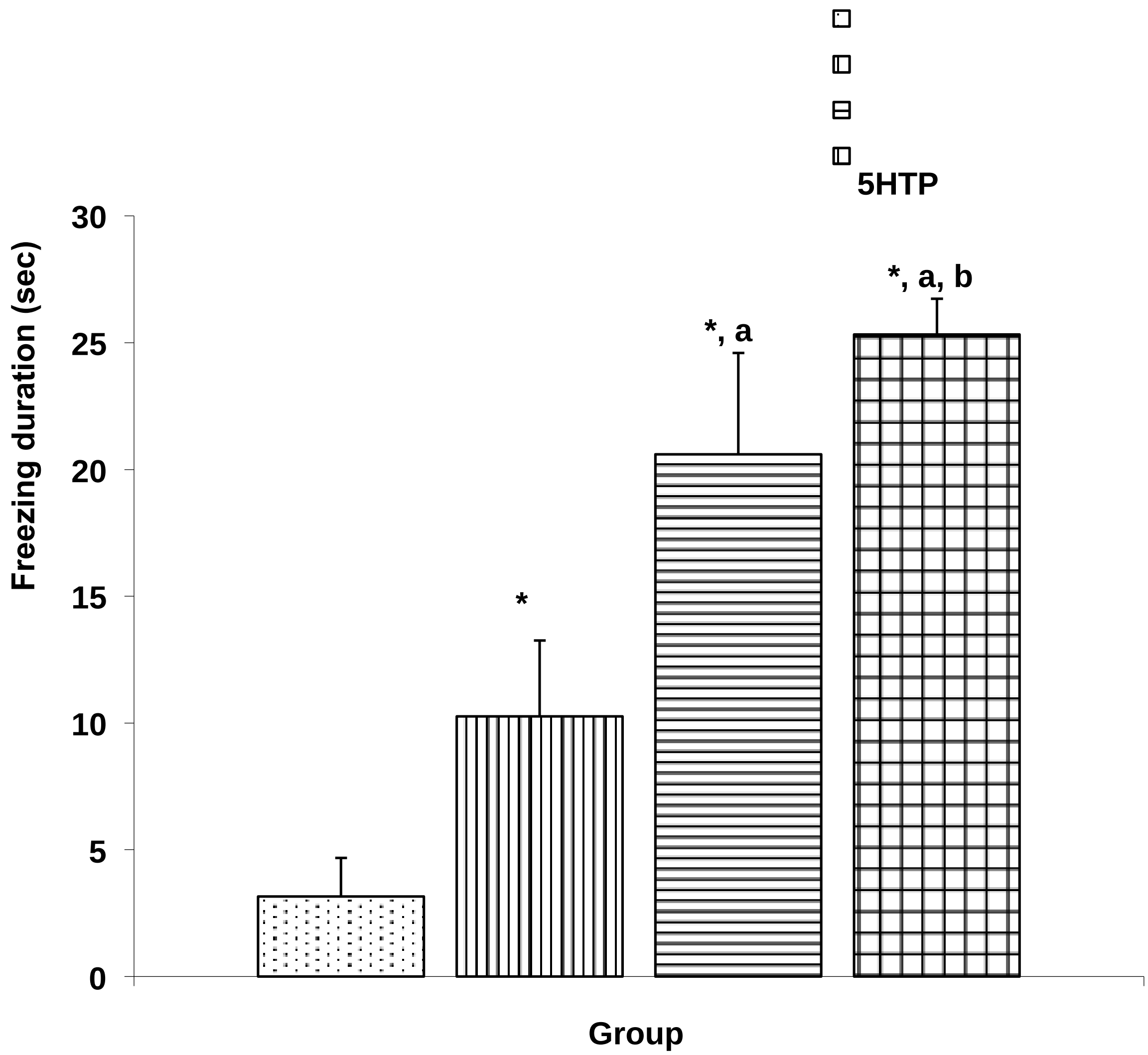

Figure 5: Freezing durations in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control; a = p<0.05 vs cooked bean; b = p<0.05 vs uncooked bean.**

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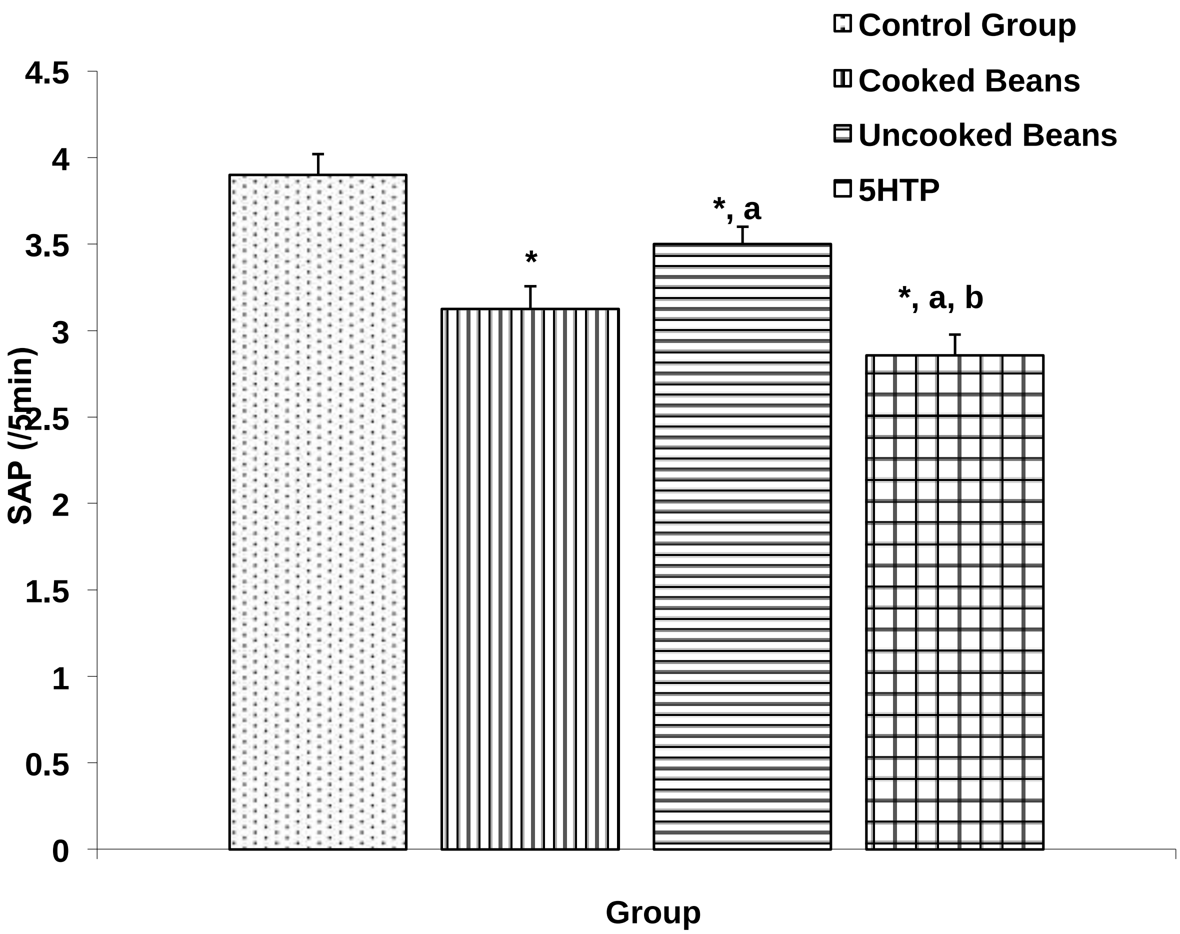

Figure 6: Frequency of stretch attend postures in the different experimental groups during the open field maze test. Values are expressed as mean ± SEM, n = 10. *p<0.05 vs control; a = p<0.05 vs cooked bean; b = p<0.05 vs uncooked bean.

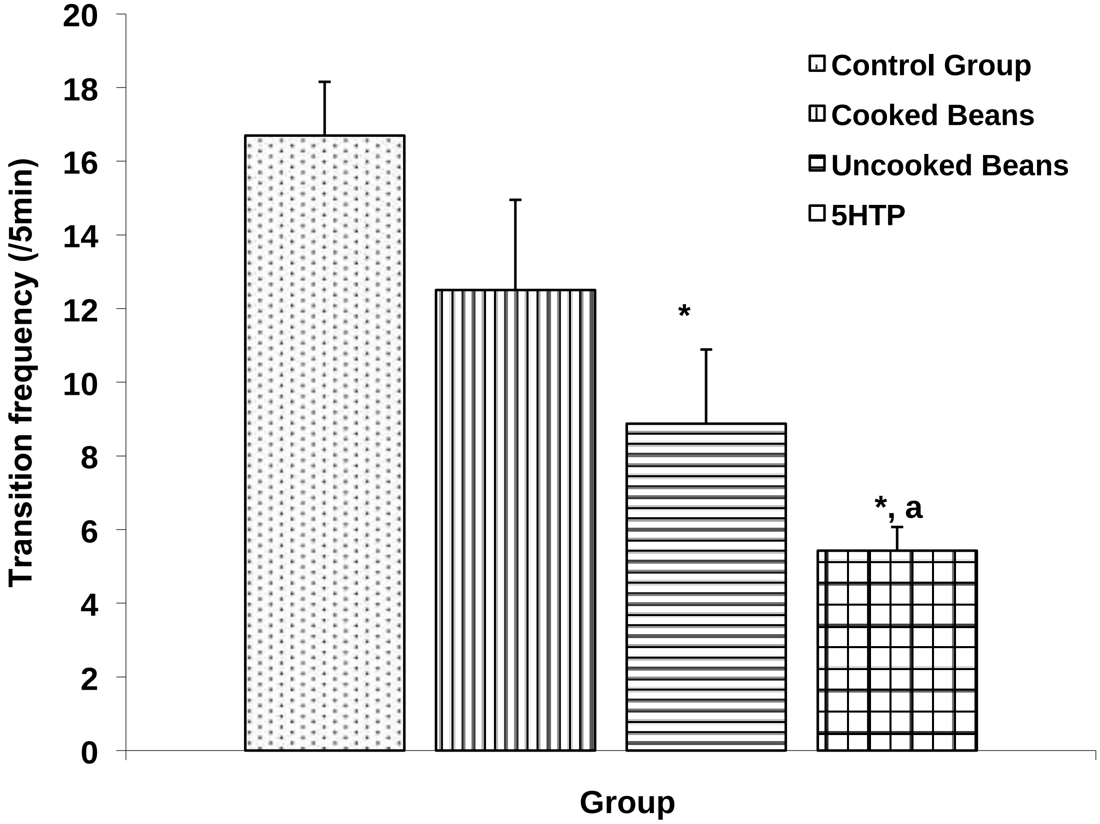

Figure 7: Transition frequencies in the different experimental groups during the light dark box transition test. Values are expressed as mean ± SEM, n = 10. *p<0.05 vs control; a = p<0.05 vs cooked beans.

DISCUSSION

In order to assess the long term consumption of black eye beans (Vigna unguiculata) diet on locomotor behaviour in mice, the open field apparatus and the light and dark transition box (LD) were employed. This method is in line with Brown *et al.*, (1999), Archer (1973), Rodgers (1997) and Streng (1974), who used the open field apparatus to assess the locomotory and exploratory behaviour of animals in a novel environment. The locomotor behaviours scored in this study included line crossing, rearing, stretch attend posture, frequency of freezing, duration of freezing and frequency of wall.

In the open field test, behaviours such as the number of line crosses and the frequency of rearing are used as measures of locomotor activity, and as a measure of exploration and anxiety. A high frequency of these behaviours (line crossing, rearing) indicates increased locomotion and exploration. Other open field maze parameters indicate anxiety and fear as well as exploration. In the open field test, behaviours such as the number of line crosses and the frequency of rearing are used as measures of locomotor activity, and as a measure of exploration and anxiety. A high frequency of these behaviours (line crossing, rearing) indicates increased locomotion and exploration. Other open field maze parameters indicate anxiety and fear as well as exploration. In the open field test, the number of lines crossed following long term consumption of cooked and uncooked beans diet was significantly decreased compared to the control. The frequency of line crossing in the group of mice that consumed the serotonin precursor diet was also decreased compared to the control group. This indicates a lower locomotor and exploratory activity in the cooked, uncooked and serotonin precursor group of mice and higher in the control group. Similarly, walling frequency was significantly reduced in the beans and serotonin precursor fed mice and higher in their frequency and duration of freezing when compared to control. These observations, shows that beans diet reduced locomotor and exploratory activities in mice. In the light/dark transition box, a similar result, like that of the open field was observed. The line crossing frequencies of the cooked, uncooked and serotonin precursor observed groups were significantly lower compared to control. This indicates a lower locomotor activity in the cooked, uncooked and serotonin precursor groups and higher in the control group. This means that locomotor and exploratory activities in the cooked and uncooked beans was reduced probably because of serotonin and some yet unknown constituents in beans(cooked and uncooked) which have impaired the mesencephalic locomotor region that control the central pattern generators in the spinal cord responsible for locomotion .This may be so because drugs e.g. L-Dopa that excite this region stimulate locomotion and vice versa(Osim,2008).It is also conceivable that cooked or uncooked beans inhibits other motor areas of the nervous system, notably the motor cortex, cerebellum or spinal cord. The frequency of rearing was not significantly different in the four experimental groups when compared.

The black eye beans have been shown to contain serotonin (5-HT) which is a neurotransmitter that can induce sleep (Brunton *et al.*, 2005). It also contains the precursor of serotonin, 5Hydroxytyptophan (5-HTP) which is converted into 5-HT.It is possible that beans diet may have increased the biosynthesis of serotonin in the brain of the mice which in turn caused the reduced locomotor and exploratory activities. Our findings therefore suggest that the long term consumption of beans diet decreases locomotion and exploratory behaviour.

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