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Research Article

Effects of beans (*Vigna unguiculata*) consumption on anxiety and fear in Swiss white mice

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ABSTRACT

Beans, the staple diet of Nigerians contains serotonin and its precursor, 5-HTP in significant amount. It was therefore the aim of this study to find out whether long term consumption of cooked beans (*Vigna unguiculata*) diet has effects on some neurobehavioral parameters notably; anxiety and fear. Thirty (30) adult Swiss white mice (18-30g body weight), were used for the study. The elevated plus maze (EPM) was employed for the evaluation of anxiety and fear related behaviour. Mice in the control group (n=10) were fed normal rodent chow while mice in the cooked beans (n=10) and serotonin precursor fed group (n=10) were fed cooked beans diet (50%w/w) and (0.2mg/50g w/w) serotonin precursor diet for 4 weeks. All animals were allowed free access to clean drinking water. Daily food intake, water intake and body weight change were measured. In EPM, the duration in the open arm and centre square duration was significantly higher ($p<0.001$ and $p<0.05$ respectively) in the test group, while the frequency of grooming and defecation was lower in the test group ($p<0.05$ and $p<0.001$ respectively). Signifying a decrease in anxiety and fear, this was also observed in their head dips. There was also a significant ($p<0.05$) decrease in the duration of grooming in the elevated plus maze test for the cooked beans and serotonin precursor fed group when compared to the control. Thus, chronic consumption of cooked beans diet may decrease anxiety and fear related behavior.

INTRODUCTION

Common bean (*Vigna unguiculata*) is a dicotyledon and belongs to the pea family (Gatel, 1994). Beans are available as dry mature seeds or the green immature seeds which are wrapped in pods (Wortmann, 2006). Both types are edible. There are many varieties of dry bean classes depending on colour, shape and size. Some of the commonly consumed varieties are navy, black, kidney and pinto beans. Bean are used as staple food in Nigeria and globally (Wader et al., 1998) and are a superb source of protein, carbohydrates, dietary fibre, minerals, vitamins and many phenolic compounds (Adeyere, 1995). Bean is a very nutritious food (Shansuddin and Elsayed,1998; Van der poel et al., 1990b),and has been reported to exhibit anticarcinogenic, anti-mutagenic (Gref*and* Eaton, 1993); anti – inflammatory, anti-diabetic, hypoglycaemic, depurative, cardio-protective and antioxidant effects (Bennick*et al*., 2008); however, different types of beans have varying content of serotonin and its precursor 5-Hydroxytrytophan (5-HTP) (Portas*et al.,* 2000).They also contain saponins, tannins, glycosides and flavonoids (Portas et al., 2000). Akey featureof serotonins is the regulation of neurobehavior such as mood, memory, learning, and sleep (Brunton *et al*.,2005).Evidence suggest that serotonin acting as a neurotransmitter on both neurons and muscles is able to modulate behavior in response to changing cues, to affect egg laying, pharynxgeal pumping, locomotion in the roundworm *Ceanorhabditis elegans* (C.elegans) (Daniel and Micheal, 2007). Since beans contain serotonin and 5-HTP and chemicals that can potentially affect behavioral patterns, it may be worthwhile to investigate

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whether long-term consumption of cooked 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 (Messman, 2005). Therefore, this study explores the effect of long term consumption of cooked beans on behavior (using mouse behavioral model).

MATERIALS AND METHODS

Experimental animals/grouping:

Adult Swiss white mice(n=30) 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 three groups (10/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±3ºC and 40-70% respectively with 12-hour natural light-dark cycle. The animals in the control group (1) received normal rodent feed (rodent chow) only, while the test group received mixed feed of 50g of cooked dried beans per every 50g of rodent chow making 50% of the beans diet (test group 2) and (0.2mg/50g) serotonin precursor diet (group 3) for 4 weeks. For the purpose of this work, we are comparing only three groups (i.e. control, cooked beans and the serotonin precursor group) instead of the four groups as shown in the results**.**

Experimental Design:

The Elevated Plus maze was made of wood-block and consisted of four arms (39.5cn long x 10cm wide) fixed to a cenral platform(10cmx10cm): two had 12 high walls(closed-arms) and two had no border in place of the walls(open-arms). The maze was elevated to a height of 40cm.

Procedure:

The mice were brought individually to the testing room 1 hour before the test was started. For testing, the mice was placed in the centre square of the elevated pus maze facing the open arm. Behaviour was recorded by a group of experimental, unaware of the treatments for a period of 5 minutes. The primary measurements were the number of entries and the time spent in each arm, head dips, stretch-attend postures, grooming, defecation, freezing and rearing etc.

Statistical Analysis:

Data collected were expressed as Mean ± SEM (standard error of mean), analysis of variance (ANOVA) and the Tukey's multiple comparison tests (Post hoc test) were used for detailed analysis. All analyses were carried out using GraphPad Prism 6 software. "P" value less than 0.05, was considered statistically significant. Note that, * signifies P<0.05; ** signifies P<0.01; *** signifies P<0.001 while 'ns' signifies not significant.

RESULTS

The following paradigms were evaluated as means of behavioral changes.

Behaviours scored in the elevated plus maze (EPM).

Frequency of open arm entry

The frequency of open arm entry for mice fed control, cooked beans and serotonin precursor diets was observed to be 4.20± 0.63; 3.75± 0.63; and 5.29± 0.63/5mins respectively. The frequency of open arm entry for mice fed cooked beans and serotonin precursor was not significantly different compared to the control group (Fig.1).

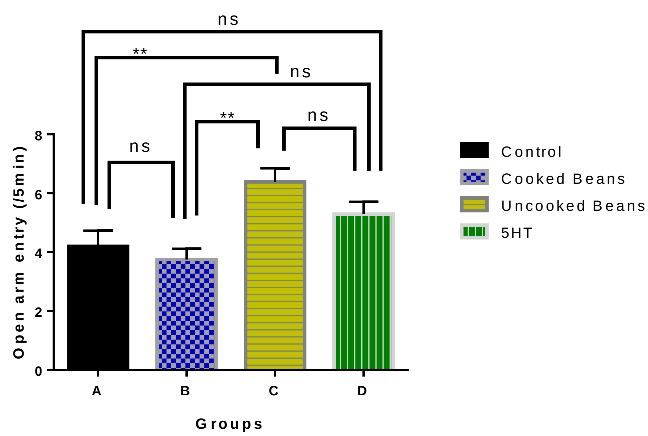

Fig.1: Open arm entry in the different experimental groups during the elevated plus maze test.

Open arm duration

The duration (time) the animals spent in the open arm of the apparatus for mice fed control, cooked beans and serotonin precursor diet was recorded as 96.62± 11.13; 186.27± 5.93seconds and 201.26± 5.40seconds respectively. The graph in figure 2 shows that mice fed cooked beans and serotonin precursor was significantly higher (p<0.001) than that of the mice fed control diet (Fig. 2).

Frequency of centre square entry

Centre square entry following long term consumption of control, cooked beans and serotonin precursor diets were found to be 3.40 ± 0.48; 2.50 ± 0.33; and 7.43 ±

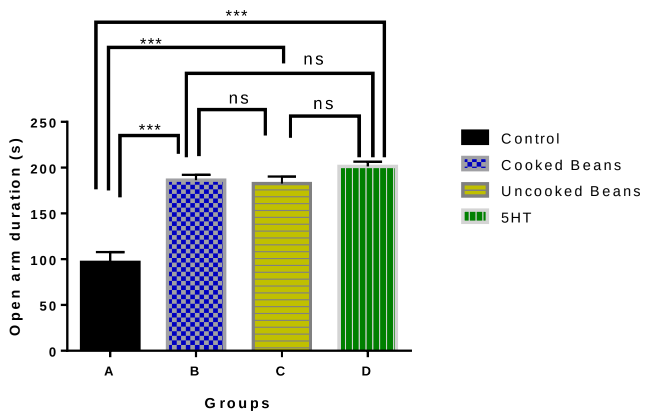

Fig. 2: Open arm duration in the different experimental groups during the elevated plus maze test.

0.65/5mins respectively. It was observed that the frequency of centre square entry was statistically higher in the group of mice fed serotonin precursor diet (P<0.001) compared to control. However, the frequency of centre square entry of the serotonin precursor fed mice was also significantly higher compared to the cooked beans group. See figure 3 for details.

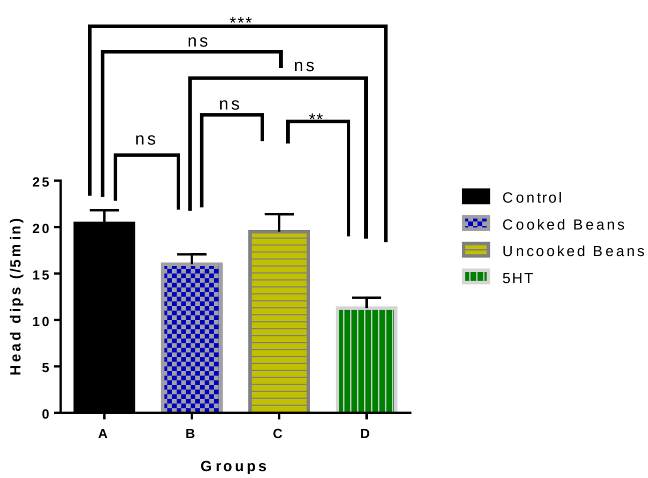

Fig 3: Head dips in the different experimental groups during the elevated plus maze test.

4. *Centre square duration*

Figure 4 shows the centre square duration of mice fed control, cooked beans and serotonin precursor diets was recorded as 28.71 ± 5.76; 25.35 ± 4.61 and 40.72 ± 3.48 seconds respectively. The centre square duration of the cooked beans and serotonin precursor fed mice was significantly higher (P<0.001) compared to control. However, the centre square duration in the group of mice fed serotonin precursor diet was significantly higher (P<0.001) compared to cooked beans group.

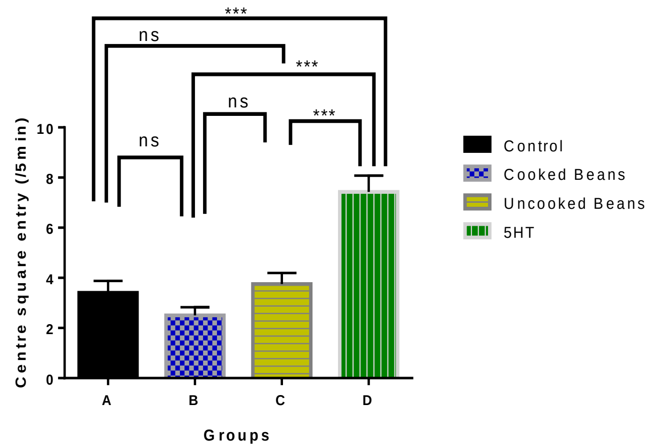

Fig. 4: Centre square entry in the different experimental groups during the elevated plus maze test.

Frequency of head dipping

The frequency with which the animal lowered its head over the sides of the open arms toward, the floor was recorded as 11.29± 2.11; 16.00± 2.08/5mins and 20.40± 2.43/5min for mice fed control, cooked, beans diet and serotonin precursor diet. Mice fed cooked beans and serotonin precursor diet showed, a higher frequency of head dips compared to control (p<0.05; P<0.01) (Fig.5).

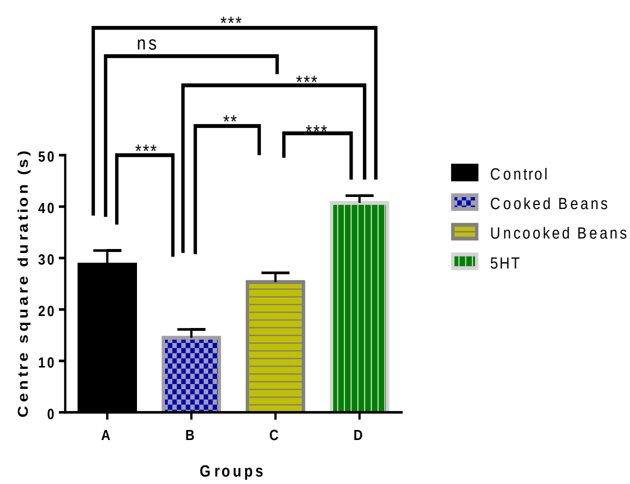

Fig. 5: Centre square duration in the different experimental groups during the elevated plus maze test.

Grooming frequency

Figure 6 shows the frequency of grooming of mice fed control, cooked beans and serotonin precursor diets were recorded as 4.20 ± 0.90; 2.63 ± 0.86 and 2.86 ± 0.40/5mins respectively. There was significant difference among the groups.

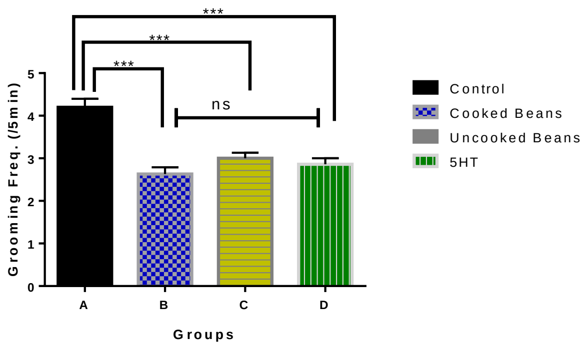

Fig. 6: Centre square duration in the different experimental groups during the elevated plus maze test.).

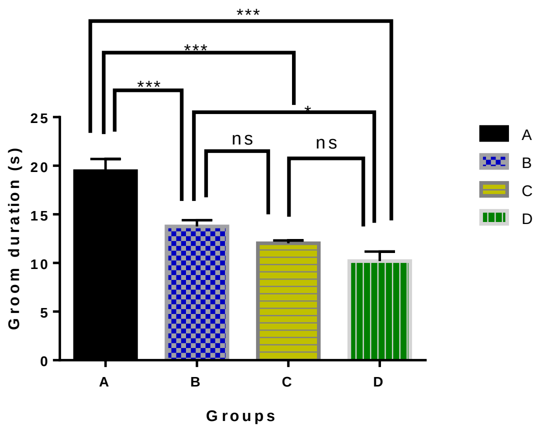

Fig. 7: Duration of grooming in the different experimental groups during the elevated plus maze test.

Grooming duration

The grooming duration shown in figure7 were, 19.44 ± 3.25; 13.76 ± 2.34 and 10.20 ± 0.97 seconds for mice fed control, cooked beans and serotonin precursor diets respectively. The grooming duration of the cooked beans was significantly lower from control at p<0.001; however, that of the serotonin precursor fed mice was significantly lower (p<0.001) compared to control.

Frequency of rearing

The frequency of rearing for mice fed control, cooked beans and serotonin precursor diets were, 20.20± 1.97; 17.38 ± 2.15 and 10.14 ± 0.70/5mins respectively. It was observed that the frequency of rearing was not significantly different in the group of mice fed cooked beans compared to control. However, the rearing frequency in the group of mice fed serotonin precursor diet was significantly lower (P<0.001) compared to control and at p<0.01 compared to cooked beans fed mice. See figure 8.

Frequency of defaecation

Fig. 9 compares the frequency of defecation in the group of mice fed control, cooked beans and serotonin precursor diets. The values are: 1.40 ± 0.52; 0.25 ± 0.16 and 0.71 ± 0.29/5mins respectively. The frequency of defecation in the group of mice fed cooked beans and serotonin precursor was significantly lower (P<0.001) compared to control.

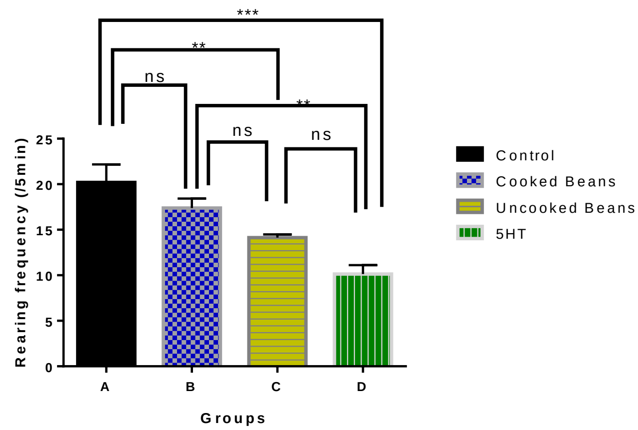

Fig. 8: Rearing frequency in the different experimental groups during the elevated plus maze test.

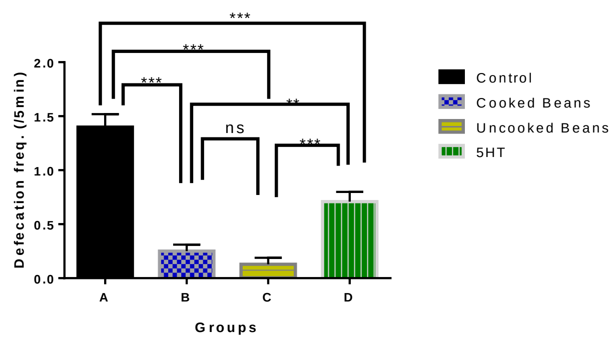

Fig. 9: Defecation frequency in the different experimental groups during the elevated plus maze test.

DISCUSSION:

The elevated plus maze (EPM) consists of two 'open arms' and two 'closed 'arms' in the shape of a plus. The open arms are aversive to mice because they are open and the maze is elevated (Lister, 1990).The closed arms provide a sense of safety because they are enclosed like most tests of anxiety (the light/dark box and open field).This task exploits the conflict between the natural tendency of mice to explore novel areas and fear of open spaces.

Behaviors such as open arm activity and head dipping are considered exploratory and a greater frequency of these measures shows a greater level of exploration. Fear behaviours include, closed arm activity, stretch attend posture, grooming frequency and duration, defecation and urination, a greater number of these measures implies a greater level of emotionality or fear,(Lister,1990).Risk assessment behaviours such as head dips and stretch attend postures are indexes of levels of anxiety. The demonstration of anxiety in this study was done using the elevated plus maze. In the elevated plus maze, the duration of grooming was observed to be lower in the cooked beans and serotonin precursor diet fed mice. Grooming is a displacement response and it is associated with anxiety in rodents when they are introduced into a novel environment (Costal *etal*., 1989). Another behavior that strongly correlates with anxiety is the closed arm duration in the elevated plus maze. This duration was found to be significantly lower in the cooked beans diet fed mice compared to control. Fearful mice would normally spend more time in the closed arms of the elevated plus maze. This also shows that the cooked beans and serotonin precursor diet fed mice showed decreased fear and anxiety compared to control. The frequency of defecation which is a measure of fear and anxiety was significantly lower for the beans group than the control values. On the other hand, the frequency of head dips for the cooked beans and serotonin precursor fed mice was significantly higher compared to the control. The open arms duration was also significantly higher than the control values. These show a lower level of anxiety and fear. Open arms duration and the frequency of head dips are both behavior which point to decreased anxiety. These behavior correlate strongly, and the higher their value, the less the anxiety level. So, cooked beans consumption may be reducing anxiety in the animals. On the other hand, open arm avoidance by rodents in the elevated plus maze gives a measure of anxiety (Trullas and Skolnick, 1993).

Fear and anxiety are basically controlled by neural circuitry involving the amygdala mostly and the hypothalamus. Other areas of the brain that may be involved in the control of fear and anxiety are the nuclei of the hypothalamus. Electrical stimulation of the amygdala for instance is associated with fear and feeling of terror in the animals (Osim, 2008). Beans are known to contain cardiac glycosides and the neurotransmitter, serotonin, etc. Cardiac glycosides reduce heart contraction(Pierce, 1996),whereas serotonin decreases tension, lessens depressive feelings and promotes the relaxation of skeletal muscle tone(Portas,2000).Thus, it is possible that the presence of these compounds and other constituents in the beans could be responsible for the anxiolytic property of bean which act by inhibiting the excitability of the amygdala by increase in the threshold of response of the cells of these nuclei, thereby reducing fear related behaviour in the mice(Costal etal., 1989; Adolph etal., 2005). These observations can also be explained by the assertion of Young &Teff (1989) that increase level of brain serotonin facilitates the calming, relaxing and mellowing serotonin neural circuits which frequently serve to counterbalance the arousing activating dopamine/noradrenaline circuit, so that anxious, agitated emotion occurs when a person's dopamine/noradrenaline activity arousal circuits are functioning strongly, without the calming, relaxing, mellowing serotonin circuits functioning strongly as a compensatory counterbalance. It is possible that those mice did not show anxiety and fear related behaviour because beans may have increased the level of brain serotonin and thus facilitated the calming, relaxing and mellowing serotonin circuits.

In conclusion, if the result of these findings is extrapolated to man, then, long term consumption of cooked beans can be used to ameliorate panic disorders and posttraumatic stress disorders.

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