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Comparative Neurobehavioural Effect of *Vigna unguiculata* decrease Anxiety Related Behaviour in Swiss White Mice

Aduema, W.,[1] Wariso, A.C[2] Agbai, J.U.[3]
[1] Department of Human Physiology, Gregory University, Uturu, Abia State
[2] Department of Human Physiology, Abia State University, Uturu, Nigeria
[3] Department of Human Anatomy, Abia State University, Uturu, Nigeria
Email: Wadioniaduema@gmail.com.
Corresponding Author: Aduema, W.

ABSTRACT
The effects of long term administration of *Vigna unguiculata* on anxiety related behavior in mice was studied. A total of 40 Swiss mice (male &female) were used for the experiment. They were randomly assigned into four groups (Control, Cooked beans, Uncooked beans and Serotonin precursor group) containing ten (10) mice each for the tests on neurobehaviour. Group 1 served as the control and was fed normal rodent chow only. Group II and Group III were fed (50% w/w) cooked and uncooked beans diet while Group IV was placed on serotonin precursor (5-HTP) diet (0.2mg/50g) respectively and clean drinking water ad libitum for 30 days. The light/dark transition box was used to access anxiety and fear related behavior. The group treated with cooked and uncooked beans had lower anxiety related activity compared to control, as seen in light chamber duration and grooming activities, etc .In conclusion, long term administration of *Vigna unguiculata* decreases anxiety and fear related activities in mice.

Keywords: Cooked beans, Anxiety, Fear, 5HTP and Mic

INTRODUCTION

Vigna unguiculata (Black eye beans) are annual plants cultivated in temperate and semitropical regions. It belongs to the pea family (Gatel, 1994). There are many varieties of dry bean classes depending on the colour, shape and size. Some of the commonly consumed varieties are black, kidney, red, navy and pinto beans. Black eye Beans are commonly known as cowpea or Iron beans in Nigeria, which are sweet in taste, soft texture and medium size and oval-shaped. Their coats are excellent source of anthocyanins and other phenolics with the potential to be used as

natural food colorants with exceptional ant diabetic potential (Mojica *et al*., 2017).They play a vital role in the vegetarian diets and provide numerous health benefits connected with eating patterns (Haddad and Tanzman, 2003)They serve as a cost effective source of nutrients (Shansuddin and Elsayed, 1998; Van der poel et *al*., 1990b) with numerous health benefits, which are generally attributed to their high protein content, dietary fibers, low saturated fat content, vitamins, minerals and phytochemicals, as well as replacement in the diet, when they substitute for animal products(Messina,2014.,Kumar *et al*., 2017.,Adeyere,1995), it has been reported that beans have anticarcinogenic, anti-mutagenic (Gref *and* Eaton, 1993; anti – inflammatory, anti-diabetic, hypoglycaemic, depurative, cardio-protective and antioxidant effects (Bennick *et al*., 2008),and are rich in serotonin and its precursor 5-Hydroxytrytophan (5-HTP) (Portas *et al*., 2000). A key feature of serotonins is the regulation of neurobehavior such as mood, memory, learning, and sleep (Brunton *et al*., 2005). Since beans contain serotonin and 5-HTP and chemicals that can potentially affect behavioral patterns and how behavioral disorders still remain a global health concern (Messman, 2005).Therefore, this study explore the effect of consumption of beans on behavior(using mouse behavioral model).

MATERIALS AND METHODS

Preparation of *Vigna unguiculata* Extract

The beans were sorted to remove debris and sand particles and then rinsed thoroughly under cool water and allowed to dry. The dried beans were then ground into fine powder with the aid on an electric blender.80g of the ground powder was then thoroughly soaked in 200ml ethanol for 48 hours at room temperature. The mixture was filtered into 250ml conical flask with Whatman filter paper number one. The filtrate was dried at a temperature of 30^0c for 10 hours to produce a gel like extract, which weighed 13.335g. Appropriate concentrations of the extract were then subsequently made by serial dilution with distilled water for further experimentation.

Animal Care

Adult Swiss white mice(n=30) weighing between 18-30g 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 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±3^{0}c and 40-70% respectively with 12 hour natural light-dark cycle. They were fed with normal rodent chow and given water freely for two weeks to allow for acclimatization before the commencement of the experiment.

Animal Treatment

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 and uncooked beans per every 50g of rodent chow making 50% of the beans diet (test group 2&3) and (0.2mg/50g) serotonin precursor diet(group 4) for 4 weeks.

Ethical Approval

All authors hereby declare that principle of Laboratory animal care was followed. All animals have been examined and approved by the appropriate ethics committee.

Procedure

Mice were carried into the test room in their home cages. Each mouse was picked by the base of its tail and placed in the centre of the white compartment facing the door and allowed to explore the apparatus for 5 minutes. The mouse behaviour were scored within the period and the maze clean with a solution of 70% ethyl alcohol and then allowed to dry between tests.

Behaviours to be scored included the following;

- **Transition:** number of times the animal passes in or out of the light and dark chambers with all four paws.
- **Light box duration**: length of time the animal spends in the light chamber.
- **Dark box duration:** length of time the animal spends in the dark chamber.
- **Stretch attends posture**: frequency with which the animal demonstrates forward elongation of the head and shoulders followed by retraction to original position.
- **Grooming**: frequency and duration of time the animal

spent licking or scratching itself while stationary.

- **Urination**: number of puddles or streaks of urine.
- **Defecation**: number of faecal bole produced.

Statistical Analysis

Numerical data was compared using bar charts with error bars and presented as mean and standard deviations. Statistical significance was assumed at P≤0.05. Data collected were expressed as Mean ± SEM (standard error of mean), analysis of variance (ANOVA) and the Turkey's multiple comparison tests (Post hoc test) were used for detailed analysis. "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 Light/Dark Transition Box

1. Light Chamber Duration in the Light/Dark Transition Box

The values for the light chamber duration are: 146:58± 8.02 (control), 167.89± 13.19 (uncooked), and 159.88 ± 12.61 (cooked) and 170.00 ± 26.02 seconds (serotonin precursor).The duration of time the animal spent in the Light Chamber in the group of mice fed cooked, uncooked beans and serotonin precursor diets was statically higher though not significant compared to control. (fig1).

2. Grooming Frequency

Figure 2 shows the frequency of grooming of mice fed control, cooked, uncooked beans and serotonin precursor diets were recorded as 2.60 ± 0.19; 1.25 ± 0.19, 2.38 ± 0.19 and 1.57 ± 0.19/5mins respectively. There was significant difference among the groups.

3. Grooming Duration

The grooming duration shown in figure7 were, 14.53 ± 2.78; 11.84 ± 2.78, 12.79 ± 2.78 and 1.75 ± 2.78 seconds for mice fed control, cooked, uncooked beans and serotonin precursor diets respectively. The grooming duration of the beans treated group was not significantly different from control .However, that of the serotonin precursor fed mice was significantly lower (p<0.001) compared to control.

4. Frequency of Rearing

The frequency of rearing for mice fed control, cooked, uncooked beans and serotonin precursor diets were, 16.70± 2.51; 12.50 ± 2.51.,8.88 ± 2.51 and 5.43 ± 2.51/5mins respectively. It was observed that the frequency of rearing was not significantly different in the group of mice fed beans and serotonin precursor diets compared to control.

5. Frequency of Defaecation

Figure.5 compares the frequency of defecation in the group of mice fed control, cooked, uncooked beans and serotonin precursor diets. The values are: 0.80 ± 0.22; 1.63 ± 0.22., 1.25± 0.22 and 0.2 ± 0.22/5mins respectively. The frequency of defecation in the group of mice fed cooked beans was statistically higher ($p<0.01$) compared to control.

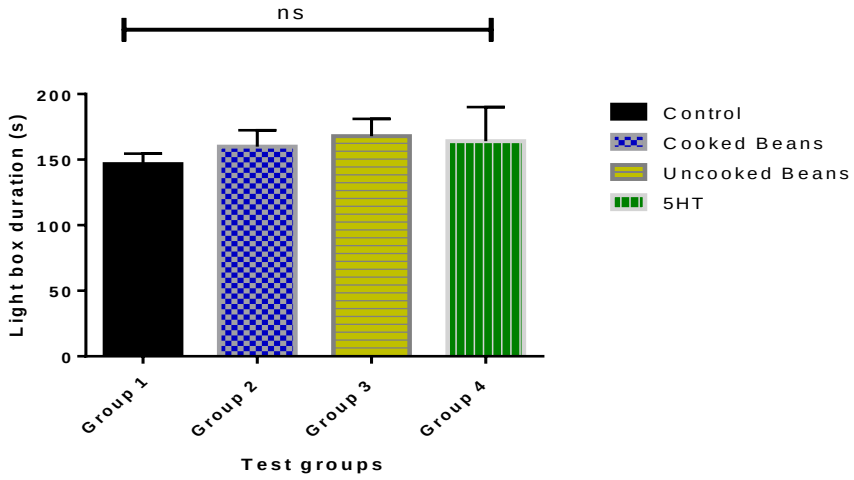

Fig 1: light box duration in the different experimental groups during the light dark box transition test. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

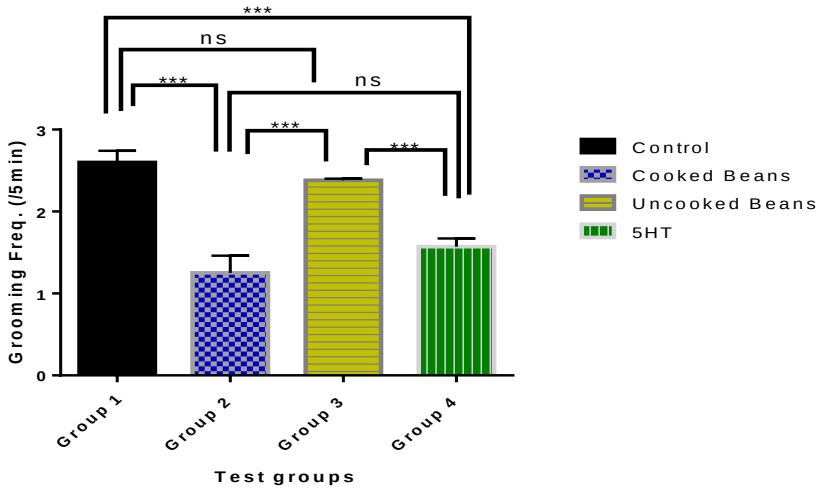

Fig.2: Grooming frequency in the different experimental groups during the light dark box transition test. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

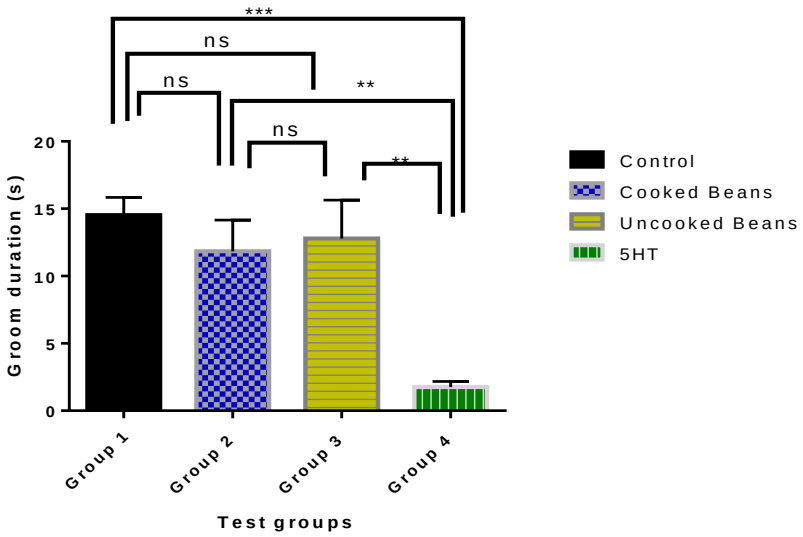

Fig.3: duration of grooming in the different experimental groups during the light dark box transition test. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

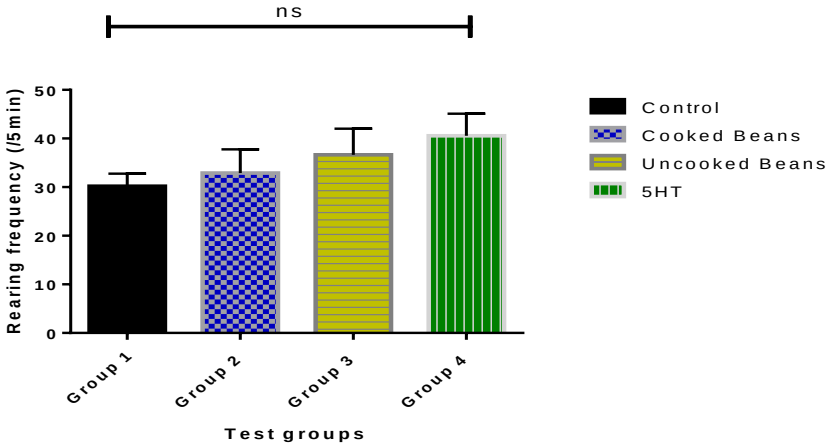

Fig.4: rearing frequency in the different experimental groups during the light dark box transition test. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

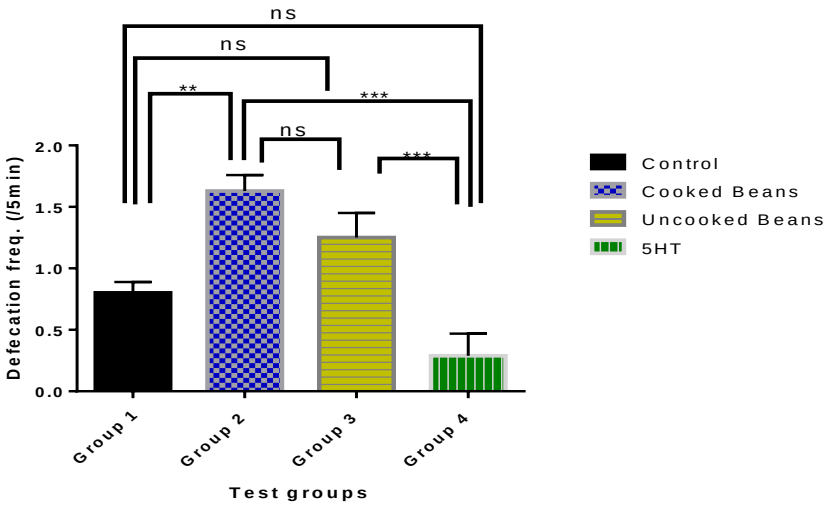

Fig.5: defecation frequency in the different experimental groups during the light dark box transition test. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

DISCUSSION

In the light and dark transition box, rodents are well known to spend less time in well illuminated surrounding and this is exactly what the mice do. In the light and dark box experiment, behaviours such as frequency of grooming and

defecation in the light/dark chamber was observed to be lower in the beans and the serotonin precursor diet fed mice compared to control. However, the serotonin precursor fed mice was significantly lower compared to cooked and uncooked beans group. This result is consistent with the observation in the elevated plus maze and it corroborates the fact that there was a decreased level of anxiety in the mice following treatment with beans and 5HTP. Similarly, the duration of grooming was significantly lowered in the beans and serotonin precursor fed mice compared to control. This indicates decrease in the level of anxiety. The duration of time spent in the light compartment by mice fed cooked, uncooked beans and serotonin precursor fed mice was statistically higher, though not significantly compared to control. However, the duration of time spent by the serotonin precursor fed mice was significantly higher than that of the beans group.

Fear and anxiety are basically controlled by neural circuitry involving the amygdala mostly and the hypothalamus. Electrical stimulation of the amygdala for instance is associated with fear and feeling of terror in the animals (Osim, 2008).Beans is 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. 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.

CONCLUSION

If the result of these findings is extrapolated to man, then consumption of beans can be used to ameliorate panic disorders and posttraumatic stress disorders.

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