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COMPARATIVE EFFECTS OF LONG TERM CONSUMPTION OF COOKED, UNCOOKED DRY BEANS (Vigna unguiculata) AND SEROTONIN PRECURSOR (5-HTP) DIETS ON NEUROBEHAVIOURAL DESPAIR, SOCIAL BEHAVIOUR AND BODY WEIGHT CHANGE IN SWISS WHITE MICE.

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ABSTRACT

Purpose: Beans, the stable diet of Nigerians contains serotonin and its precursor, 5-Hydroxytryptophan in significant amounts. It was therefore the aim of this study to find out whether long term consumption of cooked and uncooked dry beans (*Vigna uniguiculata)* diet has effects on some neurobehavioral parameters notably; neurobehavioural despair and social behaviour using Swiss white mice as experimental animals.

Method: 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 was placed on serotonin precursor (5-HTP) diet (0.2mg/50g w/w) for thirty days. All the mice had access to clean drinking water ad libitum. Before the neurobehavioral parameters were assessed, the phytochemical analysis of the beans, LD_{50} of the beans (*Vigna uniguiculata*) and that of the serotonin precursor (5-HTP) were determined. Serotonin and serotonin precursor (5-HTP) concentration were measured. Neurobehavioral despair and social behaviour were investigated alongside food and water intake and body weight change. Involvement of serotonin pathway was investigated using the set of mice administered serotonin precursor for comparison with the beans diet fed mice. Forced swim test was used to assess depression (neurobehavioral despair) and nesting score to assess social behaviour.

Results: The results showed that food intake was reduced in cooked and uncooked beans diet-fed mice when compared to control ($p<0.01$, respectively). There was no change in water intake and body weight when compared to control. The forced swim test showed no significant difference among the groups while the nestle score showed significant difference among the groups. The administration of serotonin precursor diet (5-HTP) produced similar results as cooked or uncooked beans, thus suggesting that serotonin may be involved in the action of beans on neurobehavioral parameters.

Conclusion: In conclusion, long term consumption of beans diet does not affect depression (neurobehavioral despair), body weight change and water intake but reduced food intake and improves social behaviour. One of the active chemicals involved in these effects following beans consumption may be serotonin.

Keywords: *Nestle cotton, Beans, Depression, Body weight and mice.*

1.0 INTRODUCTION

Bean offers a superb source of protein, carbohydrates, dietary fibre, minerals, vitamins and many phenolic compounds (Adeyere, 1995). Nowadays, researchers are particularly interested in the high antioxidant activities observed in beans. Bean is a very nutritious food from many aspects and it is not surprising that nutritionists would characterize beans as a nearly perfect food (Shansuddin and Elsayed,1998; Van der poel *et al.,* 1990b). 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). It has also been reported that beans contain serotonin and its precursor 5-Hydroxytrytophan (5-HTP) (Portas *et al.,* 2000). 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, pharyngeal pumping, locomotion and learning(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 beans diet can affect behaviour. 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). Therefore, this research on beans (*Vigna unguiculata*) is worthwhile for the public to know whether Nigeria beans will be beneficial or harmful in the listed neurobehavioral parameters, namely; neurobehavioural despair and social behaviour.

1.1 MATERIALS AND METHODS

Experimental animals: 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.

Experimental Design:

Mice were weighed using digital weight balance. Identification of animals was simply done using identification cards attached to each cage, because animals were singly housed. The mice were grouped into four: Each of these groups consisted of ten (10) mice [group 1=control, group 2=cooked beans, group 3=uncooked beans and group 4= 5HTP]. In all, forty (40) mice were used for the experiments and the experiments were run for thirty (30) days. The mice were aged between 30 and 35 days and weighed between 15g and 30g. All the animals were clinically and andrologically examined and confirmed to be free from systemic disorders.

1.2 PREPARATION OF FEED

Ten cups of bean were bought, out of which 5 cup was cooked, air dried and grounded into powder form. The uncooked bean was milled into powder form as well. Fifty gram of powdered cooked or uncooked beans was mixed separately with 50g of normal rodent chow making 50 % (w/w) of beans diet. The diet was then used to feed the test groups.

1.3 PREPARATION OF SEROTONIN PRECURSOR DIET

Serotonin precursor (5-Hydroxytryptophan) was obtained from May and Baker United Kingdom, and used for the study. From the estimation of the powdered 5-Hydroxytryptophan (serotonin precursor) content of cooked and uncooked beans according to the method of Feldman and Lee (1985); as modified by Mosienko *et al.* (2012). The serotonin precursor diet was prepared by mixing 40mg (0.04g) of the precursor in hundred gram (100g) of the feed.0ne gram (1g) of the mixture was mixed with 99g of the feed. So, that the amount of 5HTP added was equivalent to that contained in the beans diet. An electric blender was used to blend the mixture to form the serotonin precursor diet.

Nesting procedure:

Nesting behaviour has been used as an assay for social behaviour (13). Mice was housed individually and tested in their home cages. One hour before giving the mice nesting materials, all enrichment objects in the home cages of the mice were removed. About 3.0g of nesting material was supplied to each mouse in its home cage and allowed for 24 hours. Twenty hours later, the nest was assessed using the rating scale supplied by Deacon (14). This was based on what will be seen.

Nesting behaviour rating scale

Table 1

Rating	Requirements
1	Nestlet not noticeably touched (90% or more intact)
2	Nestlet partially torn (50-90% intact)
3	Nestlet mostly shredded, often no identifiable nest site, 50-90% shredded, also, less than 50% remains intact, but less than 90% is within a quarter of the cage floor (i.e., not gathered into a nest site but spread throughout cage)
4	An identifiable, but flat nest, more than 90% of the nestlet is torn, the nest is uneven, material is gathered into a nest within a quarter of the cage floor, but the nest is flat with walls higher than mouse body height for less than 50% of its circumference
5	A (near) perfect nest, more than 90% of the nestlet is torn, nest is fairly even, the nest is a crater, with walls higher than the mouse body for more than 50% of its circumference

PROCEDURE FOR FORCED SWIM TEST

This protocol as developed by Porsolt *et al.* (1977a,b) was used to test for neurobehavioural despair in mice**.** Each mouse was picked up from their home cages in a plastic container with holes in the bottom to let out water, and were individually dropped (placed in, head downward, trying to ensure that the mouse's head does not go underwater) into the glass cylinder and observed for immobility for one 5min trial. After the trial was completed, each of the mice were scooped up from the glass cylinder with the plastic container and placed in a holding cage filled with paper towel to dry for several minutes before being returned to their home cage**.**

Statistical Analysis

Data between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Post-hoc using Newman-Keuls. Data were presented as Mean ± SEM and a "P" value less than 0.05, was considered statistically significant.

2.0 RESULTS

BEHAVIOURS SCORED IN THE FORCED SWIM LATENCY TEST

FLOAT LATENCY

Figure 1, shows the float latency between the four groups of mice which are: 103.79 ± 6.20; 118.21 ± 8.96; 104.05± 3.68 and 118.88 ± 7.05 seconds for mice fed with control, cooked, uncooked beans and serotonin precursor diets respectively. There was no significant different among the groups. This is represented in figure 1.

DURATIOIN OF FLOAT

The duration of float recorded among the different experimental groups during the force swim latency test are: 196.20 ± 6.21; 181.79 ± 8.96; 196.40 ± 3.40 and 178.83 ± 7.35 seconds for mice fed with control, cooked, uncooked beans and serotonin precursor diets respectively. The difference was not significant among the groups. This is represented in figure 2.

FREQUENCY OF DEFAECATION

The number of faecal bole recorded among the different experimental groups during the swim latency test are: 3.70 ± 0.60; 3.88 ± 0.44; 3.00 ± 0.46 and 3.29 ± 0.68 seconds for mice fed with control, cooked, uncooked beans and serotonin precursor diets respectively. There was no significant different. This is shown in figure 3.

NESTING SCORE:

NESTING SCORE IN THE SOCIAL BEHAVIOUR TEST.

The nesting score in the social behaviour test of nest building was 2.30 ± 0.37; 2.38 ± 0.32; 3.00 ± 0.38 and 2.71 ± 0.42 (%) for mice fed control, cooked, uncooked beans and serotonin precursor diets respectively. There was significant differences among the groups (figure 4.).

MEAN BODY WEIGHT CHANGE

Fig 5 shows the mean body weight changes in the experimental groups. The mean values were 4.01±0.65; 5.28 ±1.47; 5.38 ± 1.34; and 3.96±1.51 grams in mice fed control, cooked, uncooked beans and serotonin precursor diet respectively. The body weight changes did not differ significantly from that of the control.

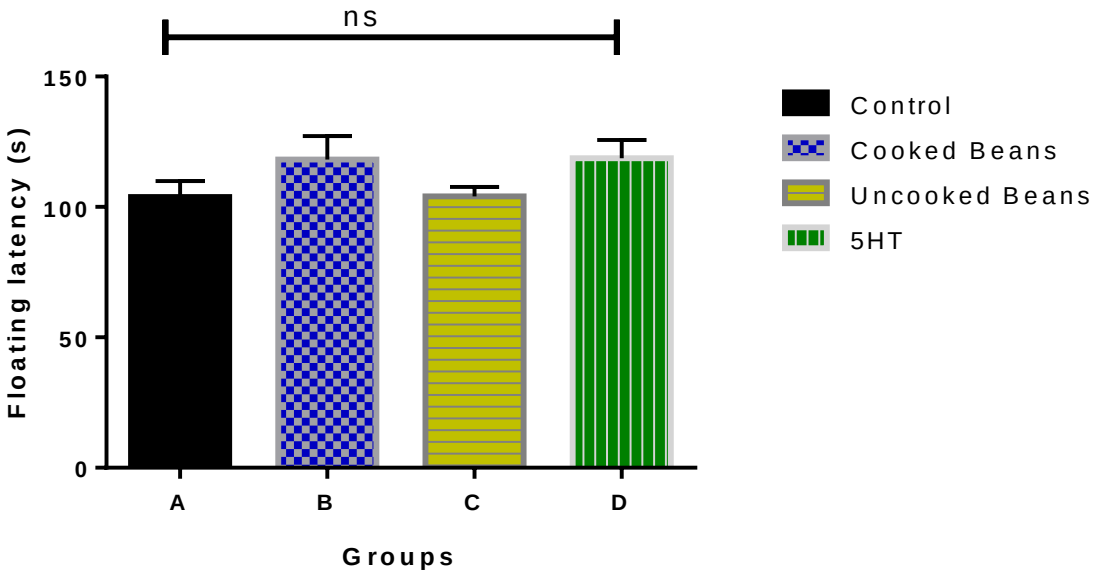

Fig1: floating latency for assessing depression among the different experimental groups. . Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

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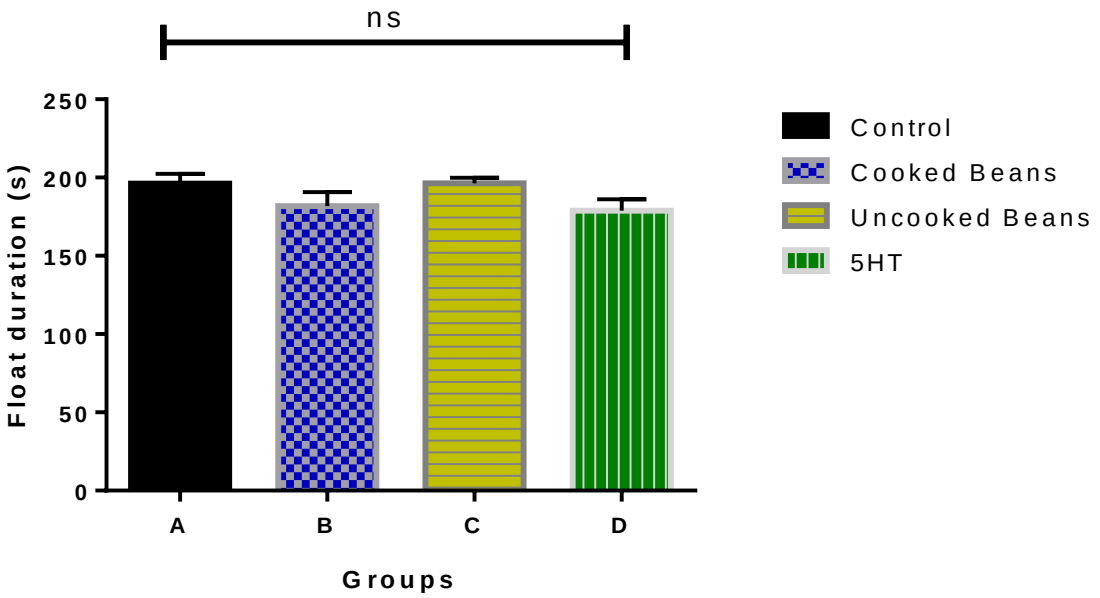

Fig2: float duration for assessing depression among the different experimental groups. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

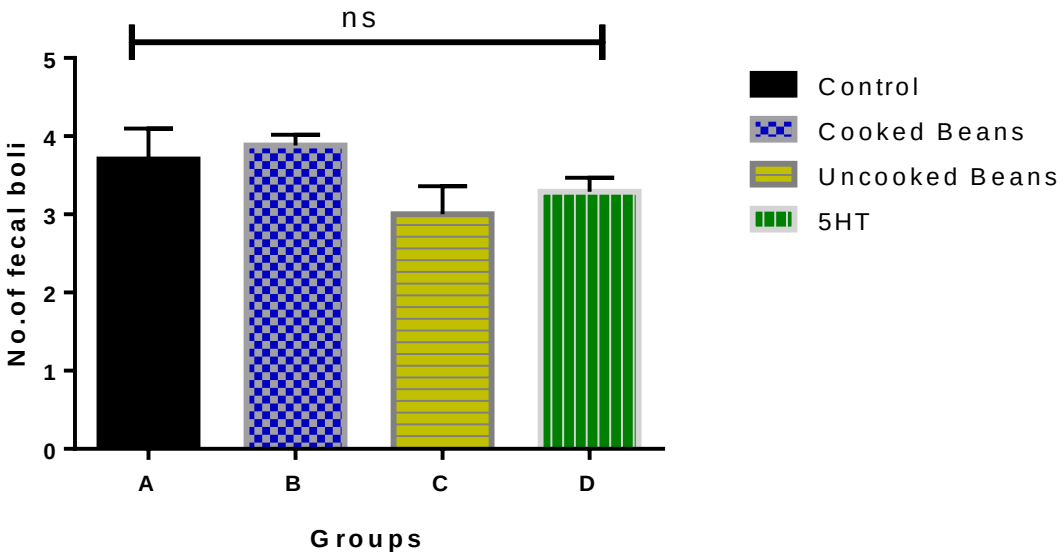

Fig3: number of fecal boli for assessing depression among the different experimental groups. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

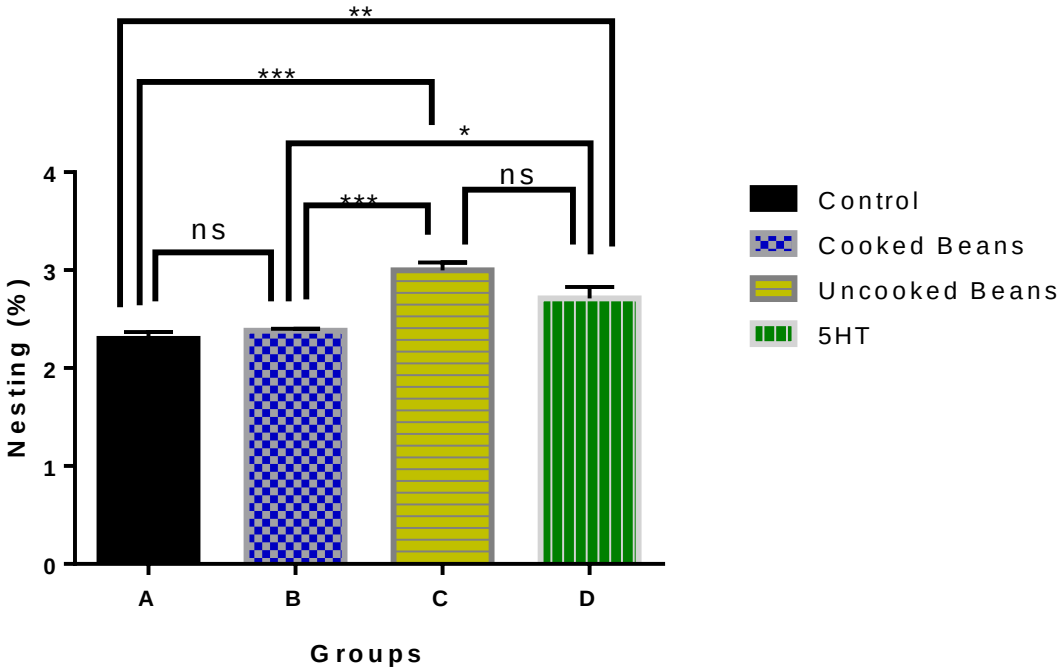

Fig4: nestle cotton for assessing social behaviour among the different experimental groups. Data were statistically analysed using One-way Anova with Tukey's multiple comparison test (Post hoc).

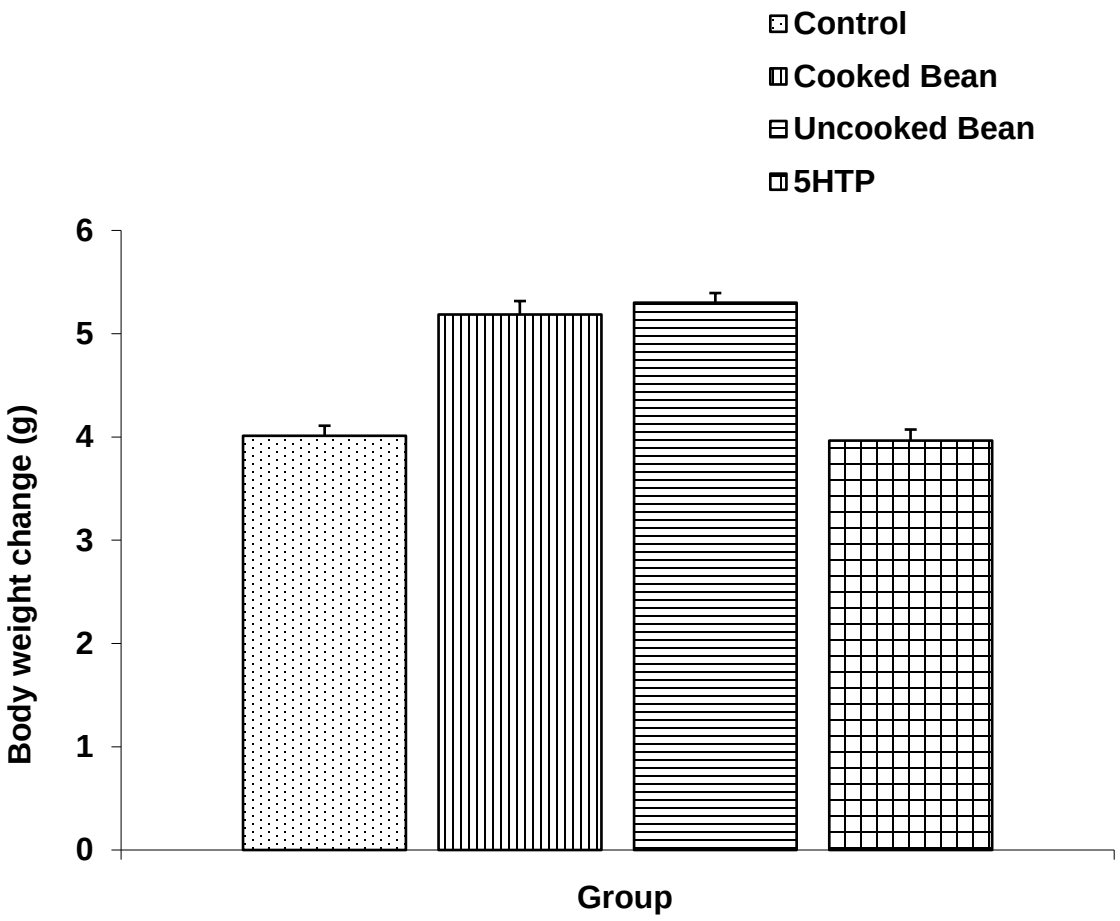

Figure 5: Body weight changes of the different experimental groups. Values are expressed as mean ± SEM, n = 10.
No significant statistical differences among groups.

3.0 DISCUSSION

The nesting score is an assessment of social behaviour. Nesting behaviour which is a reflection of social behaviour in mice may shed light on some significant disorders of human social behaviour such as schizophrenia and autism. Abnormal social behaviour exhibited in mice form a core deficit associated with autism spectrum disorder. (Delorey *et al*.,2008). Mice in this case huddle together and are able to fluff up suitable beds from their nesting materials(Bisong *etal*.,2011).A poor performance in the nesting task may indicate impairment of social relationship in the mice and perhaps a pointer to the presence of autistic behaviour. High level of nesting behaviour as indicated in the nesting score (increase grades) indicates increased social behaviour.

The results showed that the mice that were fed cooked and uncooked beans and those that were fed serotonin precursor diet were significantly different from that of the control. However, all the mice fed cooked, uncooked beans and serotonin precursor diets were able to build their nest well with no one showing any deficit in nest building. Therefore, cooked and uncooked beans diet affect social behaviour and social interaction in mice. Serotonin and beans diet may therefore affect social behavior in mice.

The forced swim test in mice was developed to test rodents for immobility because it was discovered that rodents became immobile after an initial swimming activity in an inescapable situation. The duration of immobility is considered a measure of despair or depression. The forced swim test showed no significant difference in the four (4) experimental groups when compared. The results obtained showed that the mice in the different experimental groups showed level of despair or depression when the duration of their immobility was considered. Therefore, consumption of beans diet and serotonin precursor diet does not affect depression in mice. The mean body weight change did not differ significantly in the test groups at the end of the experiments. This observation is consistent with the earlier study carried out by Livesey *et al. (*2008) which reported that eating beans helps to normalize body weight.

In conclusion; this research has shown that beans diets may have increased the serotonin levels in the brain, thus facilitating neurobehavioural processes mediated by serotonergic pathway. The current scope of this investigation suggests that long term consumption of beans diet improves social behavioral changes but induces no changes in neurobehavioural despair (depression). It also does not affect body weight. If these results are applicable to man, black eye beans consumption could be used in the control and management of ataxia, the animal model of Parkinson's disease, combat hunger and regulate body weight in obese persons.

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