MAYFEB Journal of Biology and Medicine- ISSN 2560-6948
Vol 1 (2017) - Pages 30-42

NEUROBEHAVIOURAL ACTIVITY IN MICE IN THE OPEN FIELD MAZE TASK FOLLOWING LONG TERM INGESTION OF COWPEA DIET

*Aduema W., 1 Gregory University,Wadioniaduema@gmail.com
Vidona, W.B., Gregory University,wills-bills@yahoo.com
Amah, A.K., Gregory University, amahak@gmail.com

Abstract: **It was therefore the aim of this study to find out whether long term consumption of cooked and uncooked beans (cowpea) diet has effects on some neurobehavioral parameters such as anxiety and fear using Swiss white mice as experimental animals, since cowpea contain serotonin and its precursor 5-Hydroxytryptophan which have neurobehavioral effects. Forty (40) Swiss mice were randomly assigned into four groups. Control (group A) were fed normal rodent chow, cooked beans (group B) received cooked beans diet (50% w/w), uncooked beans (group C) also received uncooked beans diet (50% w/w), while another set of mice was placed on serotonin precursor (5-HTP)(group D) diet (0.2mg/50g w/w) for thirty days. All the mice had access to clean drinking water. Anxiety and fear were investigated alongside food and water intake and body weight change. Involvement of serotonin pathway was also investigated using the set of mice administered serotonin precursor for comparison with the beans diet fed mice. The open field test was used to assess anxiety &fear related behaviors. The results showed that the Centre square entry and Centre square duration for the cooked and uncooked cowpea diet was statistically higher ($P<0.05$ and $P<0.01$respectively) compared to control while the grooming frequency for the cooked, uncooked and 5-HTP was significantly higher ($P<0.05$., $P<0.001$) compared to control. Duration of grooming was also significantly higher ($P<0.01$) compare to control. The frequency of stretch attend posture and defecation was lower in the test group ($p<0.05$ respectively). Signifying a decrease in the level of anxiety & fear. 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. In conclusion, long term consumption of cowpea diet causes calmness and sedation. It is therefore likely that it causes sleep, reduction of aggression and muscle tone. If the result of this finding is extrapolated to man, then, cowpea diet can be used to ameliorate post-traumatic stress disorders.**

Keywords: **Cowpea, Anxiety, open field, 5HTP and Mice.**

I.INTRODUCTION

Fear and anxiety are related and are sometimes used interchangeably, although they are slightly different. Fear is an unpleasant feeling of apprehension caused by the presence or anticipation of danger. Anxiety on the other hand, is a vague unpleasant emotional state with qualities of apprehension, dread, distress, and uneasiness. In addition it is objectless. Fear is similar to anxiety except that fear has a specific object. When some optimal level of stimulation or arousal is exceeded, one experiences anxiety. It can be an adaptive healthy response or a debilitating one. The limbic system, especially the amygdala has long been considered to be directly implicated in anxiety and fear. The amygdala is the name of the collection of nuclei found in the anterior portions of the temporal lobes in the brains of primates. Stimulation of some part of the amygdala cause patterns of rage, escape, punishment, severe pain and fear similar to rage patterns caused by excitation of the hypothalamus [1]; while bilateral ablation of the amygdala as is elucidated in the kluver-Bucy syndrome causes absence of fear, extreme curiosity about things and rapid forgetfulness, etc. Bean offers a superb source of protein, carbohydrates, dietary fibre, minerals, vitamins and many phenolic compounds [2].Beans contain other chemical compounds including saponins, tannins, glycosides,

flavonoids etc. Among these chemical compounds, serotonin has neurobehavioural actions on memory, learning, sleep and anxiety [3], [4]. It has been noted that apart from the fact that it is quite expensive to manage behavioural conditions with medication, no social or behavioural concern will just vanish through medication. There is therefore the need to explore an alternative that will not leave us with deleterious side effects. Sequel to these, Osim and his team have been investigating to find out if our common consumables (food substances) can affect our behaviour. They have shown that consumption of thermoxidized palm oil in the long term, increased fear and anxiety in animals([5],common malaria drugs such as chloroquine increase anxiety and pain perception[6],consumption of tobacco reduces anxiety[7] and pain [8] while artesunate decreases locomotion and exploration[9].It is likely therefore, that some stable foods can affect behaviour. 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 cowpea (cooked and uncooked) diet can affect behaviour.

II.MARETIALS AND METHODS

A.*EXPERIMENTAL ANIMAL/GROUPING*: Forty (40) adult mice weighing between 18-31g were divided into 4 groups. They were handled in accordance with the HIH guidelines for the use and care of laboratory animals, with 12 hours light and dark cycle throughout the experimental periods. The animals were fed normal mice chow (vital feed, Nigeria) and water, and both were provided ad libitum throughout the duration of the experiment. Freshly cooked and uncooked cowpea was obtained from Calabar market, Nigeria. They were washed to remove impurities. The bean was air –dried, crushed to fine powder. Group 1 was the control, treated with normal rodent chow while group 2, 3 and 4 which were the test groups were treated with 50% cooked and uncooked beans and (0.2mg/50g) serotonin precursor diet in the mornings(10am) and lasted for4 weeks.

B.EXPERIMENTAL DESIGN: The open field was used to access anxiety and fear related behavior [10]. 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 open field for 5 minutes. During the period of 5 minutes, behavior scored using a stop watch was Centre square entry and duration, frequency and duration of grooming, frequency of stretch attend posture and frequency of defecation, etc.

***C.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.

III.RESULTS:

CENTRE SQUARE ENTRY

Centre square entry following long term consumption of cooked, uncooked beans, serotonin precursor and control diets were found to be 1.60 ± 0.04/5min (control), 1.75 ± 0.05/5min (cooked), 2.10 ± 0.04/5min

(uncooked), and 2.43 ± 0.08/5min (serotonin precursor) respectively. It was observed that the frequency of Centre square entry was statistically higher in groups of mice fed cooked beans compared to control at ($p<0.05$).Uncooked beans was significantly different from control at $p<0.01$.The serotonin precursor diet fed mice was significantly different at ($P<0.001$) compared to control (fig .1). However, the frequency of center square entry in the group of mice fed with uncooked beans was significantly higher ($P<0.01$) compared to cooked beans while those fed with the serotonin precursor diet was significantly higher ($P<0.01$) compared to both the cooked and uncooked beans group.

CENTRE SQUARE DURATION

The Centre Square duration shown in figure 2 were, 1.89 ± 0.10/5min (control), 2.09 ± 0.09/5min (cooked), 2.29 ± 0.08/5min (uncooked), and 2.39 ± 0.09/5min (serotonin precursor).The center square duration of the cooked beans was significantly different from control at $p<0.05$.The uncooked beans and the serotonin precursor fed mice was significantly higher ($P<0.01$) compared to control. However, the uncooked beans group was statistically higher when compared to the cooked beans group while that of the serotonin precursor fed mice was significantly higher ($P< 0.05$) compared to both the cooked and uncooked beans group.

GROOMING FREQUENCY

Figure 3 compares the grooming frequency between the four groups of mice respectively. The grooming frequencies shown in figure 4.1.6 were 3.00± 0.30/5min (control), 2.50 ± 0.12/5min (cooked), 2.10 ± 0.21/5min (uncooked), and 1.75± 0.10/5min (serotonin precursor).The frequency of grooming among the group of mice administered cooked and uncooked beans was significantly lower ($P<0.05$) compared to control. However, the grooming frequency in the group of mice fed uncooked beans was statistically lower when compared to the cooked beans group ($p<0.05$).The serotonin precursor diet fed mice was significantly different from the control group at ($p<0.001$), whereas those fed with serotonin precursor diet were seen to be significantly lower ($p<0.01$) than those of the cooked beans and at ($p<0.05$) compared to the uncooked beans group. See figure 3.

GROOMING DURATION

Grooming duration as measured by the period of time the animal spent scratching or licking its body throughout the five minutes exposed to the maze. It was observed that the grooming duration for control, cooked, uncooked beans and serotonin precursor fed mice were,23.21±5.19 seconds (control), 18.13±1.76 seconds (cooked), 11.50±3.50 seconds (uncooked),and7.35±3.26 seconds (serotonin precursor)respectively. The cooked, uncooked beans and serotonin precursor fed group of mice groomed for a shorter time when compared to control ($p<0.01$).However, the uncooked beans group groomed for a shorter time when compared to the cooked beans group, whereas the group fed with the serotonin precursor groomed for a shorter time when compared to the cooked and uncooked beans group. Fig 4.

STRETCH ATTEND POSTURE (SAP) IN THE OPEN FIELD MAZE.

Figure 5 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± 0.12/5min (control), 3.13±0.13/5min (cooked) 3.50± 0.10/5min (uncooked), and 2.86± 0.12/5min (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 significantly 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.

DEFEACATION IN THE OPEN FIELD

The mean number of fecal bole produced at the end of every five minutes spent in the open field maze were, 3.00± 0.04/5min (control), 2.75± 0.05/5min (cooked), 2.38± 0.06/5min (uncooked), and 0.86± 0.14/5min (serotonin precursor).See figure 6.The frequency of defecation of the cooked, uncooked beans and serotonin precursor fed mice was significantly lower ($p<0.05$) compared to control. However, the frequency of defecation in the group of mice fed uncooked beans was statistically lower compared to the cooked beans group, whereas those fed with the serotonin precursor diet were seen to be significantly lower($p<0.05$) than those of the cooked and uncooked beans group.

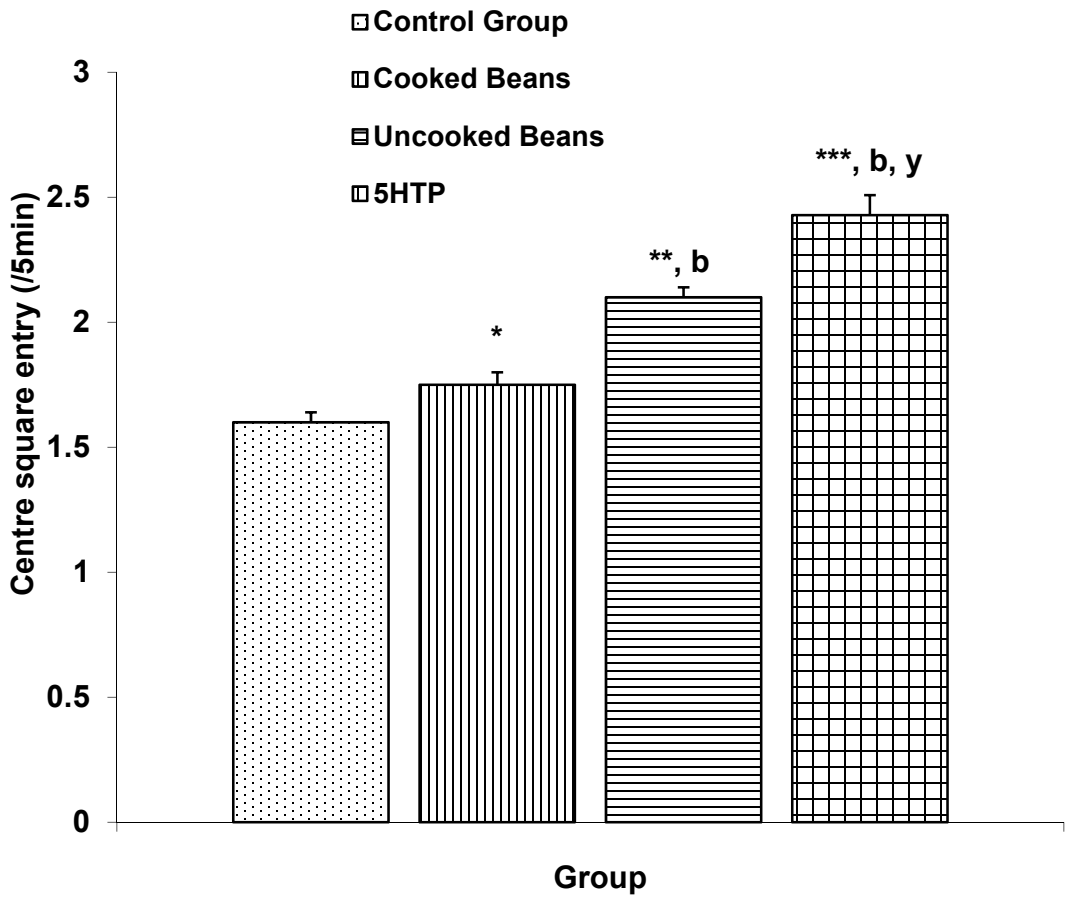

Figure .1: Frequency of centre square entry in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
p<0.05 vs control;
****p<0.01 vs control***
*****p<0.001 vs control***
b = p<0.01 vs cooked bean;
y = p<0.01

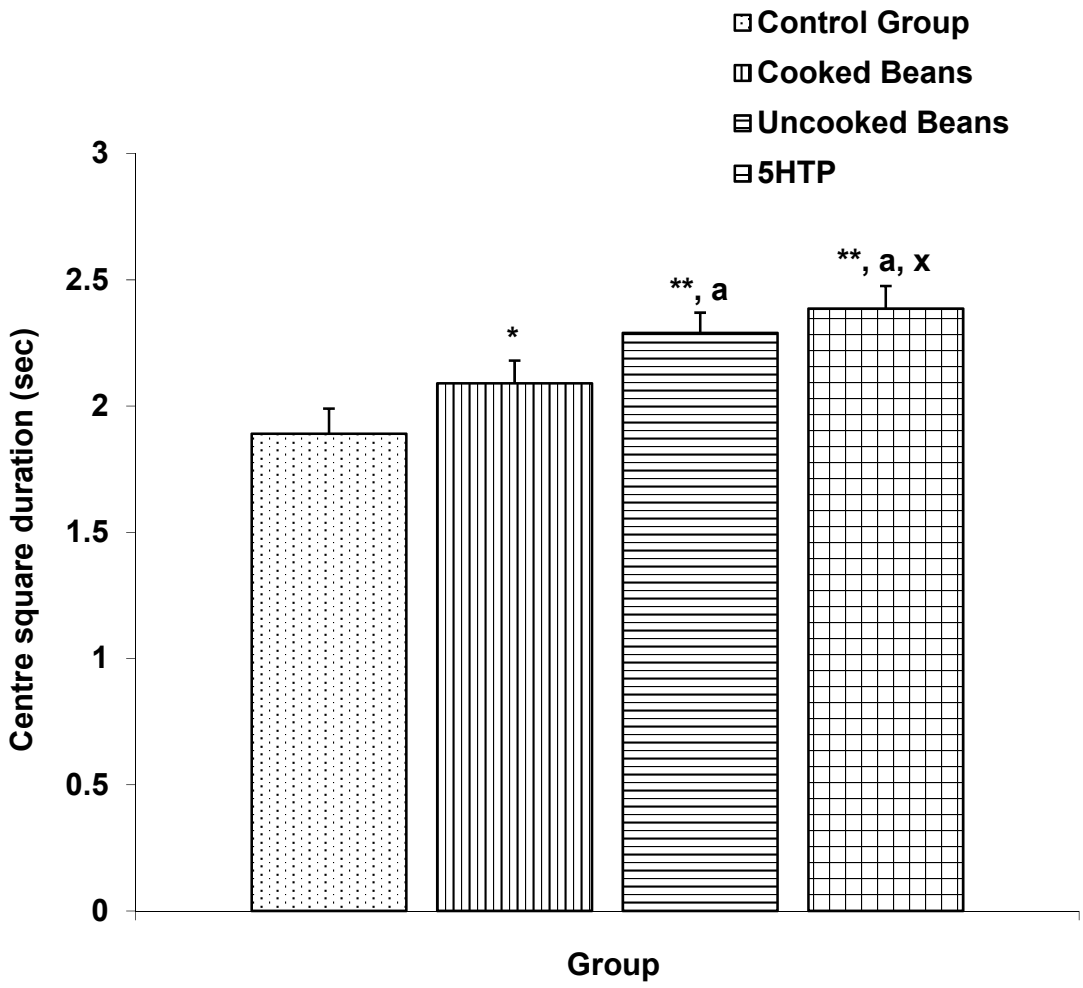

Figure 2: Centre square duration duration in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
****p<0.01 vs control**
a = p<0.05 vs cooked bean;
x = p<0.05 vs uncooked bean.

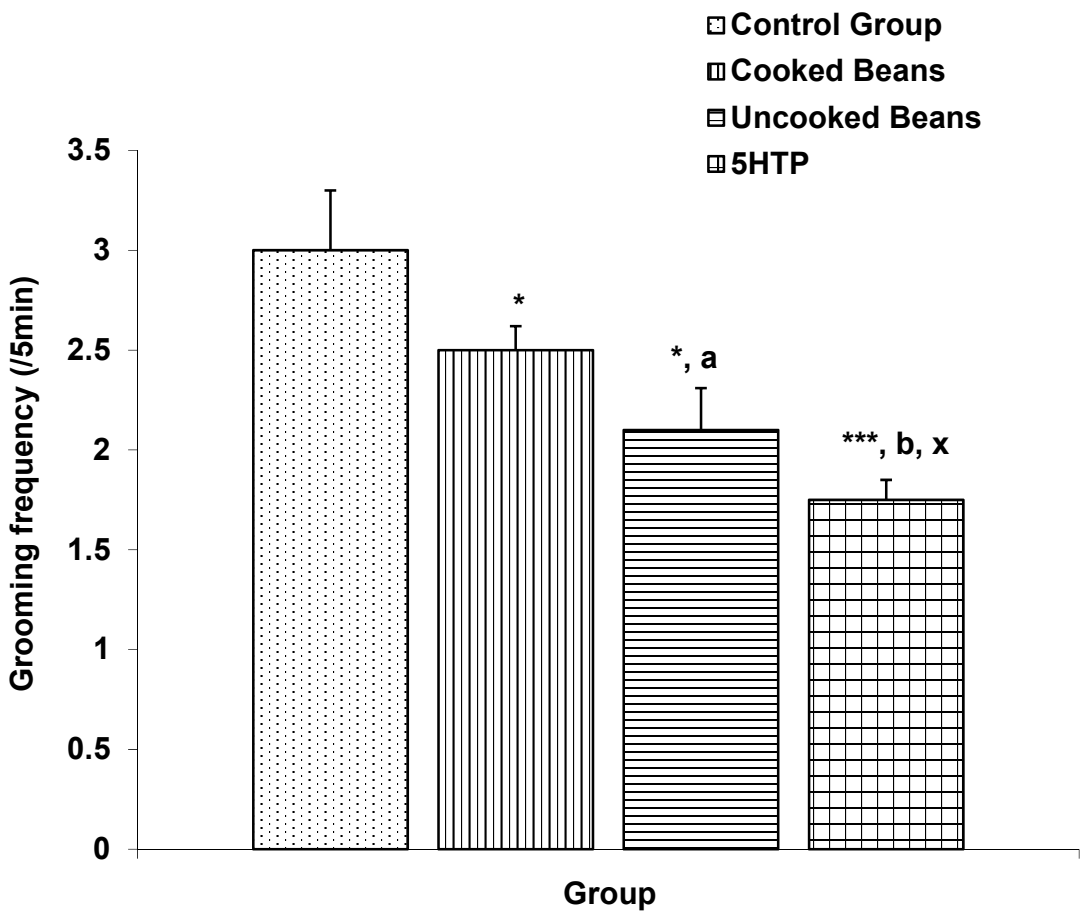

Figure 3: Grooming frequencies in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
****p<0.001 vs control**
b = p<0.01 vs cooked bean;
x = p<0.05 vs uncooked bean.

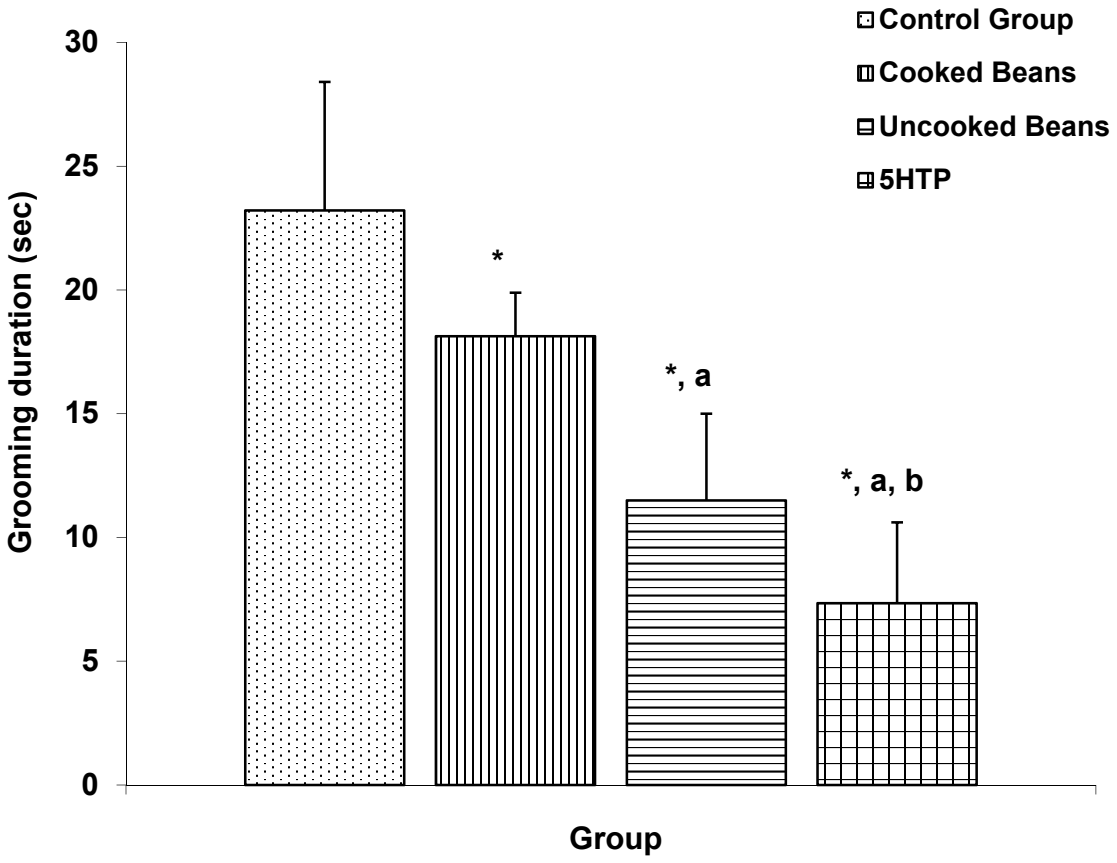

Figure 4: Duration of grooming in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
a = p<0.05 vs cooked bean;
b = p<0.05 vs uncooked bean.

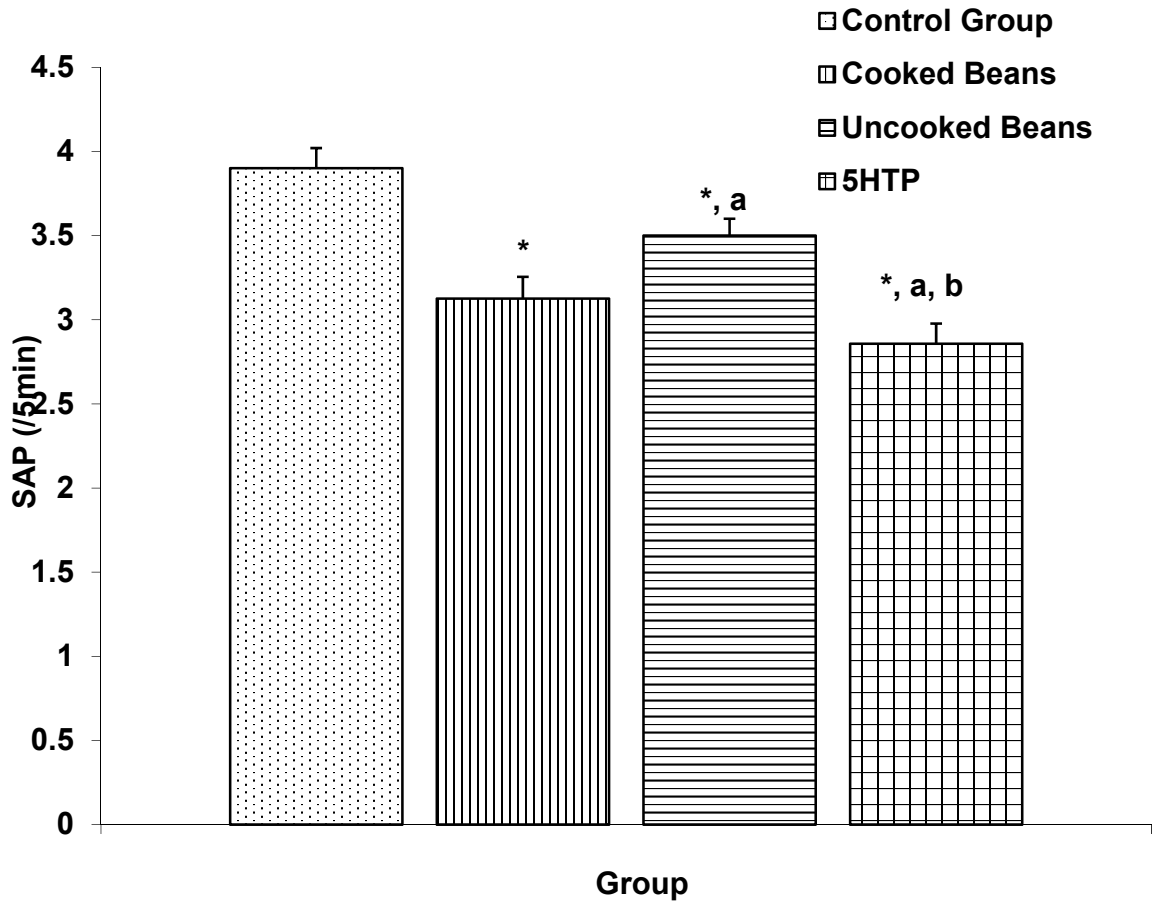

Figure 5: Frequency of stretch attend postures in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
a = p<0.05 vs cooked bean;
b = p<0.05 vs uncooked bean.

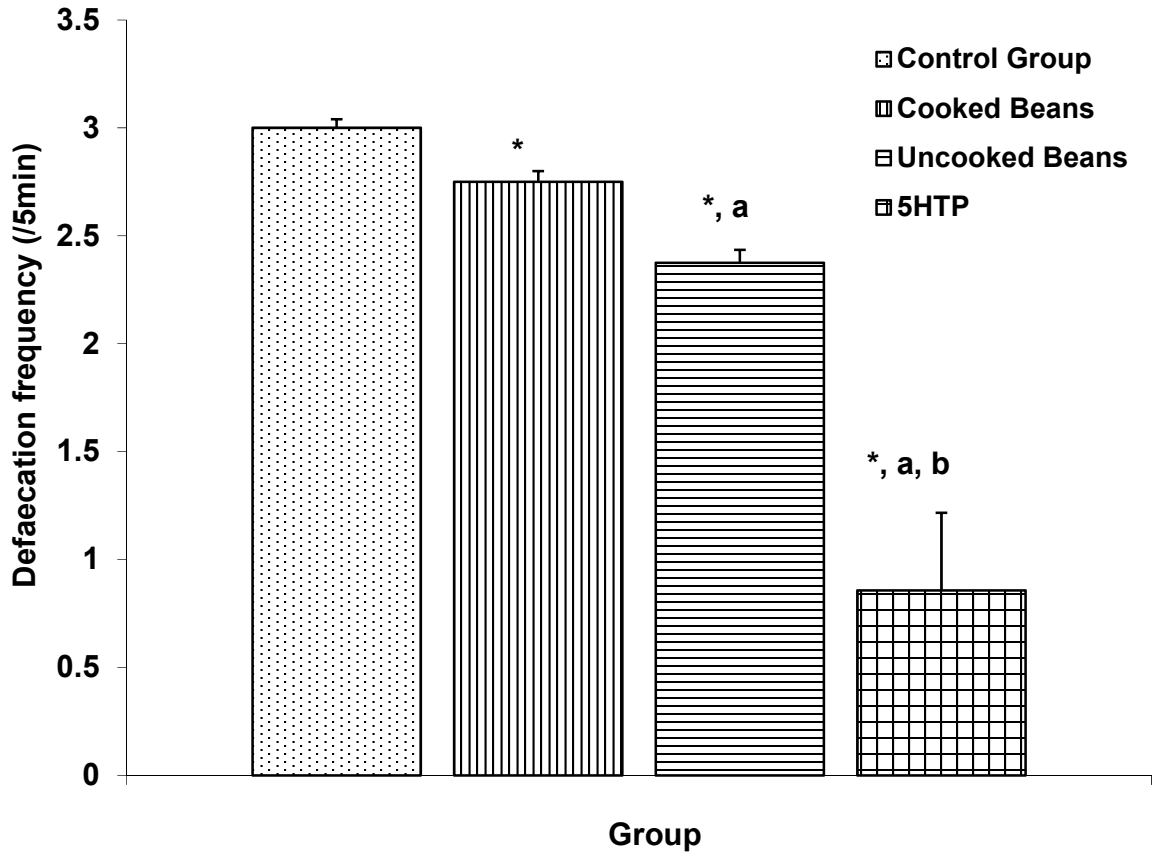

Figure 6: Defaecation frequency in the different experimental groups during the open field maze test.
Values are expressed as mean ± SEM, n = 10.
***p<0.05 vs control;**
a = p<0.05 vs cooked bean;
b = p<0.05 vs uncooked bean.

IV.DISCUSION:

The demonstration of anxiety in this study was done using the open field maze. In the open field maze, the duration of grooming was observed to be lower in the uncooked 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 [11].Another behaviour that strongly correlates with anxiety, is the Centre square duration in the open field maze. This duration was found to be significantly higher in the cooked, uncooked beans and serotonin precursor diet fed mice compared to control. Fearful mice would normally spend more time in the closed arms of the open field maze. This also shows that the cooked, uncooked beans and serotonin precursor diet fed mice showed decreased fear and anxiety compared to control. The frequency of defecation and stretch attend posture which is a measure of fear and anxiety was significantly lower than the control values. Rearing frequency is an exploratory behaviour and an increase in rearing behaviour indicates a decrease in anxiety [12].The frequency of grooming showed significant difference among the groups. On the other hand, the frequency of center square entry for the cooked, uncooked beans and serotonin precursor fed mice was significantly higher compared to the control. These show a lower index level of anxiety and fear. These behaviour correlate strongly, and the higher their value, the less the anxiety level. So, cooked and uncooked beans consumption may be reducing anxiety in the animals. 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 [13].Beans is known to contain cardiac glycosides and the neurotransmitter, serotonin, etc. Cardiac glycosides reduce heart contraction[14],whereas serotonin decreases tension, lessens depressive feelings and promotes the relaxation of skeletal muscle tone[15].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[11]; [16].

V.CONCLUSION

Our findings suggest that long term consumption of cowpea causes calmness, sleep, reduction of aggression and muscle tone. If the result of this finding can be extrapolated to humans, then, cowpea diet can be used to ameliorate post –traumatic stress disorders.

ACKNOWLEDGEMENT

We acknowledged Pa and MrsB.A.Aduema, Mr.Iwasam Joshua, Dr.Nmaju, Prof.E.E.Osim and Associate Prof.A.A.Nwankwo for their priceless support

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AUTHOR(S) CONTRIBUTION

All authors have contributed one way or the other to the success of this paper.