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RESEARCH ARTICLE

EFFECT OF COOKED BEANS (*Vigna unguiculata*) ON MOTOR COORDINATION AND SOCIAL BEHAVIOUR IN SWISS WHITE MICE

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ABSTRACT

The neurotransmitter serotonin has neurobehavioural actions which include mood, memory, learning and sleep. 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 beans (*Vigna unguiculata)* diet has effects on some neurobehavioural parameters notably; motor coordination and social behaviour using Swiss white mice as experimental animals. Thirty (30) CD1 mice were randomly assigned into three groups, viz; control, cooked beans diet (50% w/w) and 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 neurobehavioural parameters were assessed, the phytochemical analysis of the beans, LD_{50} of the beans (*Vigna unguiculata*) and that of the serotonin precursor (5-HTP) was determined. Serotonin concentration was measured in beans using gas chromatography analysis. Motor coordination 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. Beam walking was used to assess motor coordination, and the nesting score to assess social behaviour. The results showed that food intake was reduced in cooked beans and serotonin precursor 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. On the other hand, beans and 5HTP fed mice showed better motor coordination when compared to control. The nesting score showed no significant difference among the groups. Thus suggesting that serotonin may be involved in the action of beans on neurobehavioral parameters. In conclusion, long term consumption of cooked beans improves motor coordination but does not affect social behaviour, body weight change and water intake but reduced food intake. One or the active chemicals involved in these effects following beans consumption may be serotonin.

INTRODUCTION

Nowadays; there are many dry bean classes depending on the colour, shape and size of the beans. Some of the commonly consumed varieties are navy, black, kidney and pinto beans. The plant is edible for dry beans and green beans. Dry beans are the mature seeds, whereas green beans are the immature seeds wrapped in pods (1).Overall, common beans a staple food in many parts of the world (2).Bean offers a superb source of protein, carbohydrates, dietary fibre, minerals, vitamins and many phenolic compounds (3). 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 (4; 5).It is has been reported that beans have anticarcinogenic, anti-mutagenic (6) anti – inflammatory, anti-diabetic, hypoglycaemic, depurative, cardio-protective and antioxidant effects (7). It has also been reported that beans contain serotonin and its precursor 5-Hydroxytrytophan (5-HTP) (8).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 (9).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(10).Since beans contain neurotransmitters and chemicals that can potentially affect

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behavioural patterns, it may be worthwhile to find out whether long-term consumption of cooked beans diet can affect behaviour.

Therefore, this research on cooked dry beans (*Vigna unguiculata*) is worthwhile for the public to know whether Nigeria beans will be beneficial or harmful in the listed neurobehavioral parameters, namely; motor coordination and social behaviour.

MATERIALS AND METHODS

Experimental animals

Thirty (30) 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 three (3) 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 ± 3^0c 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 three: Each of these groups consisted of ten (10) mice [group 1=control, group 2=cooked beans and group 3= 5HTP]. In all, thirty (30) 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.

Preparation of Feed

Ten cups of bean was bought, out of which 5 cup was cooked, air dried and grounded into powder form.

Preparation of Powdered Beans Diet

Fifty gram of powdered cooked 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.

Preparation of Serotonin Precursor Diet

Synthetic serotonin precursor(5-Hydroxytryptophan) was obtained from May and Baker (M&B) limited, Enfield, Middle Sex, United Kingdom(UK), and used for the study. From the estimation of the powdered 5-Hydroxytryptophan (serotonin precursor) content of cooked beans according to the method of Feldman and M-Lee (11) as modified by Mosienko *et al.*, (12).The serotonin precursor diet was prepared by mixing 20mg(0.04g) of the precursor in 100g of the feed. One 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.

Procedure for motor coordination

The mice were carried to the test room in their home cages. The mouse was removed from its home cage and placed at one end of the balance beam. After the mouse has secured its grip on the beam, the trial begins. The maximum length of the trial is five minutes. The mouse is tested under white light, during the dark phase. The beam is cleaned with 70% ethanol and permitted to dry between each trial.

What was measured?

Distance travelled: The number of line crosses.
Foot Slips: Number of times one of the mouse's back feet slips from the beam

Number of turns: Frequency that the animal reversed direction
Latency to fall: Time at which the animal fell off of the beam. If a fall occurred the animal was not placed back on the beam but was returned to the home cage. The trial was not repeated.

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.

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.

RESULTS

Footslip

The frequency of foot slips of the different experimental groups was recorded as 7.20± 0.88; 4.38 ± 1.00; /5min and 2.71 ± 0.42/5min for mice fed with control, cooked beans and serotonin precursor diets respectively. The frequency of foot slips for the cooked beans was significantly different from control at ($p<0.05$., fig.1).However, the serotonin precursor fed mice was also significantly lower ($P<0.01$) compared to control.

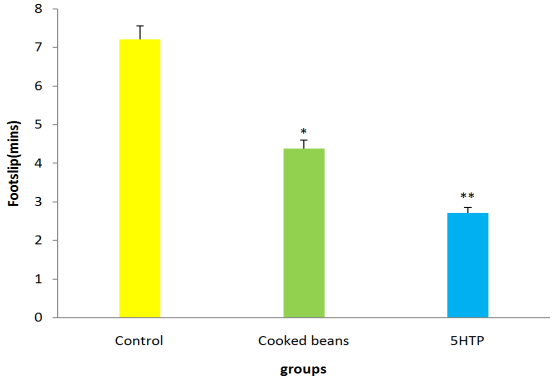

Fig 1 frequency of foot slips for assessing motor coordination among the different experimental groups. Values are expressed as mean ± SEM, n = 10; *significantly different from control at $p<0.05$; **$P<0.01$vs control.

Distance Covered

Figure 2, shows the distance covered in the different experimental groups which are recorded as: 41.71 ± 3.99; 60.88 ± 8.47/5min and 85.50 ± 8.46 for mice fed control, cooked beans and serotonin precursor diet respectively. The distance covered for mice fed with cooked beans diets during bean walking was significantly higher (P<0.05) compared to control. However, the serotonin precursor fed mice was also significantly higher (P<0.01) compared to the control group.

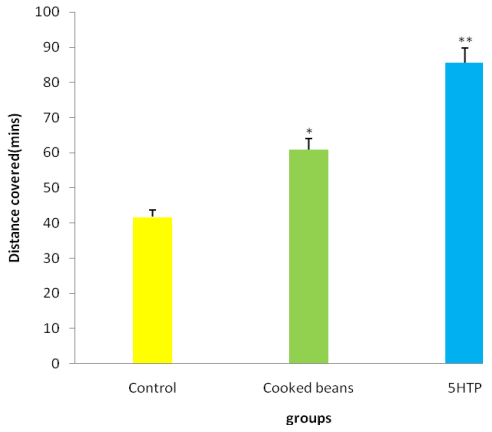

Fig 2 distance covered for assessing motor coordination among the different experimental groups. Values are expressed as mean ± SEM, n = 10; *significantly different from control at p<0.05;**P<0.01vs control.

Latency of Fall

The latency of fall of the different experimental groups is as follows: 7.36 ± 10.56; 11.83 ± 1.27 and 15.37 ± 3.48 seconds for mice fed control, cooked beans and serotonin precursor diet respectively. The latency of fall was longer for the cooked beans and serotonin precursor diet fed mice compared to control at p<0.05 (Figure.3).

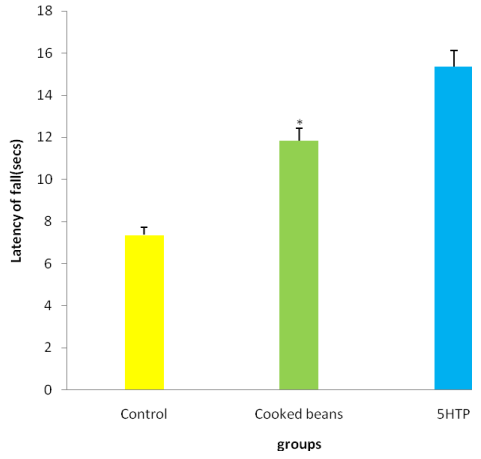

Fig3 latency of fall for assessing motor coordination among the different experimental groups. Values expressed as mean± SEM,*significantly different from control at p<0.05; **P<0.01 vs. control.

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(%) and 2.71 ± 0.42 (%) for mice fed control, cooked beans and serotonin precursor diet respectively. There was no significant differences among the groups (figure 4.).

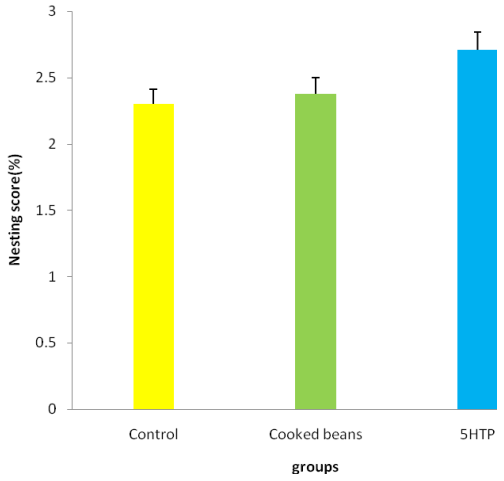

Fig 4 nesting social behaviour among the different experimental groups score for assessing. Values are expressed as mean ± SEM, n = 10; No significant differences among groups.

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 and 3.96± 1.51grams in mice fed control, cooked bean and serotonin precursor diet respectively. The body weight changes did not differ significantly from that of the control.

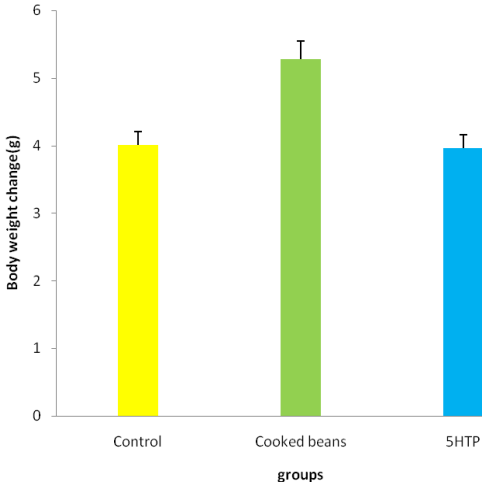

Fig 5 body weight changes of the different experimental groups. Values are expressed as mean ± SEM, n = 10; No significant differences among groups.

Mean daily food intake

Fig .6 shows the mean daily food intake values of the three experimental groups were 8.44 ± 0.5 (control), 7.9± 0.09 (cooked) and 7.9± 0.08 grams (serotonin precursor).The mean food intake was significantly lower in the group of mice fed cooked beans and serotonin precursor diet compared to control (p<0.01).

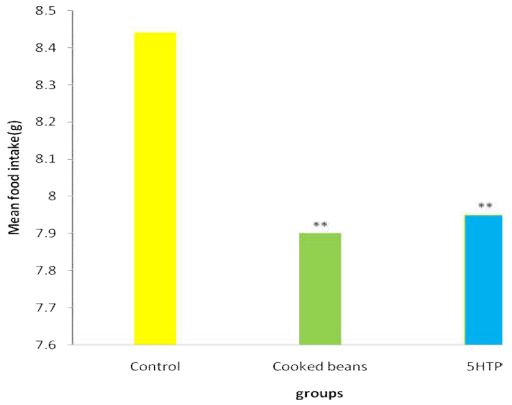

Fig 6 mean food intake of the different experimental groups.Values are expressed as mean ± SEM, n = 10; **P<0.01vs control.

Mean water intake

The mean water intake for the mice fed with control and cooked beans diet respectively; are statistically shown in fig 7. There mean values are: 5.74± 0.1 (control); 5.71± 0.13(cooked beans) and 5.61± 0.11ml (serotonin precursor). The mean water intake between the different groups was not significantly different compared to the control.

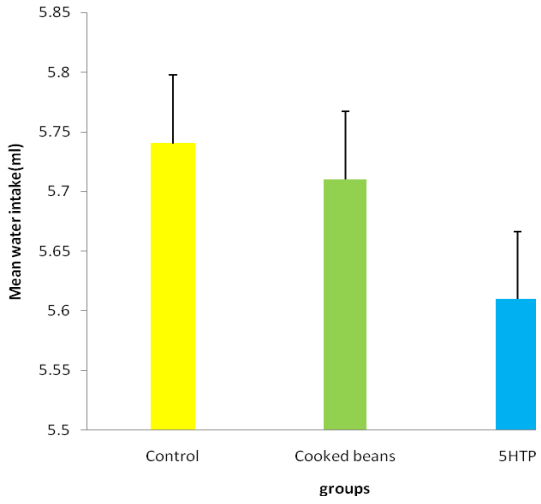

Fig 7 mean water intake of the different experimental groups. Values are expressed as mean ± SEM, n = 10; No significant differences among groups.

DISCUSSION

Beam walking is a test for motor coordination and balance. The results in beam walking showed that the beans and serotonin precursor group showed better motor coordination compared to control. This is because, decreased frequency of foot slips and longer latency of falls indicate a higher level of maneuverability in the beam, thus indicating better motor coordination and therefore the better the motor learning ability.
The mean daily food intake was observed to be significantly lower in mice fed cooked beans and serotonin precursor diet than in the group fed with the control diet. Food and water intake is controlled by the lateral hypothalamic nucleus and the ventromedial hypothalamic nucleus. The lateral hypothalamic nucleus is the hunger centre and so, when stimulated the animal eats and drinks voraciously. On the other hand, ventromedial hypothalamic nucleus is the satiety centre and so, when stimulated the animal stops feeding. It is possible therefore, that cooked beans diet may have a stimulatory effect on the ventromedial hypothalamic nucleus thus causing satiety, thereby decreasing food intake. In support of the result of this study, previous research shows that serotonin modulates dopamine action, thereby decreasing appetite (15).

On the other hand, the mean water intake was not significantly different between the test groups and the control group. Water intake is controlled by the Osmoreceptors or 'thirst' receptors in the hypothalamus. The Osmoreceptors stimulate thirst when the blood concentration of electrolytes (osmolarity) is high (16).Conversely; inhibition of this centre reduces thirst. It is likely therefore that certain unknown phytochemical compound in beans may be responsible for the regulation of the thirst centre. The exact mechanism by which cooked black eye beans reduces food intake but does not affect water intake remain to be elucidated and by implication a need for further studies in this regard.

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.,*(*17) which reported that eating beans helps to normalize body weight.

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. (18).Mice in this case huddle together and are able to fluff up suitable beds from their nesting materials(19).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 beans and serotonin precursor diet were not significantly different from that of the control. However, all the mice fed cooked beans diet were able to build their nest well with no one showing any deficit in nest building. Therefore, cooked beans diet did not affect social behaviour and social interaction in mice. Serotonin and beans diet may therefore not affect social behavior in mice.
In conclusion; this research has shown that cooked 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 (cooked) diet improves motor coordination but induces no social behavioural changes. It also decreases food intake but does not affect body weight and water intake.

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