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EFFECTS OF LONG-TERM ADMINISTRATION OF COOKED BEANS (Vigna unguiculata) DIET ON LEARNING AND MEMORY IN MICE.

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ABSTRACT

Purpose: 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; learning and memory, using Swiss white mice as experimental animals.

Method: Thirty (30) CD1 mice were randomly assigned into three groups, viz; (10, control, 20 Test) weighing 15-30g were fed normal rodent chow, 50% cooked beans diet (w/w) respectively 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) were determined. Serotonin concentration was measured in the beans using gas chromatography analysis. Learning and memory, was 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. Mice were tested in the Morris water maze for 8 days: 3 days of acquisition training, 3 days of reversal training, 1 day of single probe trial and 1 day for visible platform task.

Results: Learning on days 1, 2 & 3 of acquisition training was improved in the cooked beans and serotonin precursor diet-fed mice when compared to control ($p<0.001$, respectively). The trend was similar during the reversal training, memory was improved in the beans and serotonin precursor diet-fed mice when compared to control ($p<0.001$). During the probe trial, the swim duration in the South-East quadrant was significantly higher for cooked beans and serotonin precursor group compared to control ($p<0.05$). However, during the visible platform task, the swim latencies for the cooked beans and serotonin precursor group was significantly lower compared to control. Our finding therefore suggests that long term consumption of beans diet enhances learning and memory.

Conclusion and Recommendation: In conclusion, long term consumption of cooked beans diet enhances learning and memory. If this research can be extrapolated to humans, we recommend that the serotonergic potential of beans may be harnessed in the prevention, treatment and management of behaviour/ mental disorders as this could potentially open up a vista for improved mental health status in populations globally.

Keywords: *Serotonin, 5-Hydroxytryptophan, Learning & Memory, Beans, Vigna unguiculata, Mice.*

1.0 INTRODUCTION

Common bean (*Vigna unguiculata*) is a dicotyledon and belongs to the pea family (Gatel, 1994).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 (Wortmann, 2006).Overall, common beans a staple food in many parts of the world (Wader et al., 1998).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 is 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 (5HTP) (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 cooked 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).Human behaviour is believed to be influenced by endocrine and nervous system. The complexity in the behaviour of an organism is correlated to the complexity of its nervous system. Thus, organisms with more complex nervous systems (like the human) have a greater capacity to learn new responses and adjust their behaviour. This behaviour is influenced by physical and psychological changes that result from a complex state of feeling described as emotion (Cacioppo & Gardner, 1999).

We are aware that physiologically, to bring some emotion/behaviour under control is difficult, perhaps, owing to the paucity in connection between the limbic system(the part of the brain that controls our emotion) and the neocortex (the part of the brain whose activity can modify emotional behaviour).Furthermore, the prolonged after discharge in the limbic system following emotional stimulation makes emotional responses to outlast their stimuli (Ganong *et al.,*2010; Osim,2012).Therefore, owing to paucity of connections between the neocortex and the limbic system as well as the prolonged after discharge of the limbic system after stimulation, it is difficult to control our emotions.

In a series of talk presented at a conference, Osim(2012) noted that many people have devised various ways to help them control their emotion. They have explored methods such as music, yoga, exercise, drugs, alcohol, and religion all of which are believed to affect emotional state in one way or the other. Also 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(Osim,2012),common malaria drugs such as chloroquine increase anxiety and pain perception(Lelei *et al.*,2012),while artesunate decreases locomotion and exploration(Davies *et,al.*,2013).

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 beans (cooked) diet can affect behaviour.

1.1 MATERIALS AND METHODS

Experimental animals/grouping:

Twenty (20) 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 two (2) 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 two: Each of these groups consisted of ten (10) mice [group 1=control, group 2=cooked beans and group 3= 5HTP].In all, twenty (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.

1.2 PREPARATION OF FEED

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

1.3 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 group.

1.4 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 (1995) as modified by Mosienko *et al.*, (2012).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:

Testing in the Morris Water Maze lasted for eight days. The first three days were acquisition training with the invisible platform. Day 4-6 were reversal training, again with an invisible platform. On the seventh day, a probe trail was conducted with no escape platform. On day eight, four trials were conducted using the visible platform.

ACQUISITION (Platform in North West for days 1, 2, 3)

During acquisition training, the water was adjusted appropriately such that the platform was covered by 1cm of coloured water (invisible platform).The platform was placed in the centre of the North West quadrant. Each animal received 4 trials of 60 seconds (max) per day. The starting positions of the animals were predetermined which prevented any sequence of two trials to be repeated by the same animal during any other day.

Possible start positions were at the boundaries of the quadrants (e.g. West, North, East or South).Each mouse was removed from its holding cage using a small clean 500ml plastic container to minimize handling stress. The animal was then placed into the water at the appropriate start position, facing the centre of the pool. The mouse was then permitted to explore the pool and to search for the hidden escape platform for 60 seconds. When the animal located the platform, the timer was stopped and the animal removed using the plastic container and placed in the holding cage .If the animal did not find the platform during the allotted time, the animal was guided onto the platform using the plastic container. Once on the platform, the mouse was permitted to visually explore its surroundings for 20 seconds, at which point it was picked up in the plastic container and returned to the appropriate holding cage.

The next mouse was then placed in the pool and the same procedure followed .Each animal completed four trials per day over 3 days, i.e.12 trials of acquisition training.

REVERSAL (Platform in South East for days 4, 5, 6)

Reversal training began on day 4.The invisible platform was moved to the opposite quadrant (southeast quadrant), and the mouse again assigned to appropriate start positions. The same procedures as in acquisition training were carried out during reversal training. Each of the animals completed four trials per day for 3 days I.e. 12 trials of reversal training.
Probe (No platform, Day 7)

A probe trial was conducted on day 7.At this time; there was no escape platform at all-in the maze. Each animal completed one trial of 60 seconds. Each mouse was placed in the maze from one of the four possible start positions and allowed to explore the pool. The duration in each quadrant and the frequency of entry into the North-West and South-East were noted.

Visible Platform (Visible Platform at South West, Day 8)

The visible platform task was conducted on day 8.The visible platform was placed in the Southwest quadrant of the pool. The same procedures as in the acquisition and reversal training were carried out and each mouse completed four trials.

Data collection for Morris water maze:

During acquisition, reversal and visible platform test, the following behaviours are measured: (1) swim latency (time to find and mount the escape platform).

During the probe trial, the measures recorded are (1) frequency of entries into each quadrant (Northeast, Northwest, Southeast and Southwest), (2) duration of time spent in each quadrant, (3) the number of times the mouse crosses the location of the platform during reversal training (annulus reversal crossing), (4) the number of times the mouse crosses the location of the platform during acquisition training (annulus acquisition crossing).

2.0 RESULTS:

ACQUISITION TRAINING

On day 1, the swim latencies obtained showed that mice fed with control, cooked beans and serotonin precursor diet scored 18.19 ±1.2; 16.23±0.8; and 13.20±2.3 seconds respectively. In day 2, the swim latencies were 18.18±1; 14.30 ± 1.2; and 12.50± 1.2 seconds for mice fed with control, cooked beans and serotonin precursor diet respectively. For day 3 of the acquisition training, the swim latencies was 18.20 ±1.9; 14.50 ± 0.95; and 13.00 ± 0.78 seconds for mice fed with control, cooked beans and serotonin precursor diet respectively.

The swim latencies for the cooked beans and serotonin precursor group was significantly shorter than the control group during the 3 days of training ($p<0.001$., (Fig, 1).

REVERSAL TRAINING

On day 1, of the reversed training, the swimming latency for mice fed with normal, cooked beans and serotonin precursor diet was 21.30 ± 1.2; 18.30 ± 2; and 16.48±2.1 seconds respectively. During the second day of reversal training, the swimming latency was 21.00 ± 2.1; 18.10 ± 2.3; and 15.40 ± 2.2 seconds for mice fed normal, cooked beans and serotonin precursor diet respectively. For day three (3) of the reversal training, the swimming latency was 21.40 ± 2.3; 18.00 ± 0.9; and 15.80 ± 1 seconds for mice fed with control, cooked beans and serotonin precursor diet respectively.

The swim latencies for the cooked beans and serotonin precursor group was significantly lower during the reversal training compared to the control ($p<0.001$., Fig.2).

QUADRANT DURATION DURING THE PROBE TRIAL TASK (RETENTION QUADRANT)

Figure 3, compares the quadrant duration during the probe trial in the Morris water maze between the three experimental groups of mice. The duration of stay in each quadrant is shown in figure 3. During the trial, the two groups had more preference to the South-East quadrant (that bears the platform during the reversal training when compared to control).The preference to the SE quadrant

for the mice fed with cooked beans and serotonin precursor diet was statistically higher (P<0.05) compared to control.

ANNULUS ACQUISITION & ANNULUS REVERSAL CROSSINGS

Figure 4, compares the annulus acquisition and reversal crossings during the probe trial task. The values for the annulus acquisition crossings are 1.50 ± 0.22;1.38 ± 0.50 and 0.86 ± 0.43 seconds for mice fed with normal control, cooked beans and serotonin precursor diet respectively. The values for the annulus reversal crossings are 3.60 ± 0.58; 4.32 ± 0.35 and 0.86 ± 0.43 seconds for mice fed normal, cooked beans and serotonin precursor diet respectively. There was no significant difference among the groups in the annulus acquisition crossings. However, there was a significant difference in the cooked beans and serotonin precursor fed mice in the annulus reversal crossings compared to the control but higher in the serotonin precursor group when compared to the cooked beans group(P<0.05).

VISIBLE PLATFORM TASK

The swimming latency in the visual platform task was 25.59 ± 0.98; 22.09 ± 1.33 and 17.98 ± 1.20 seconds for mice fed with normal, cooked beans and serotonin precursor diet respectively. During visible platform task, the swim latencies of the cooked beans and serotonin precursor group was significantly lower (P<0.05., fig.5) compared to control.

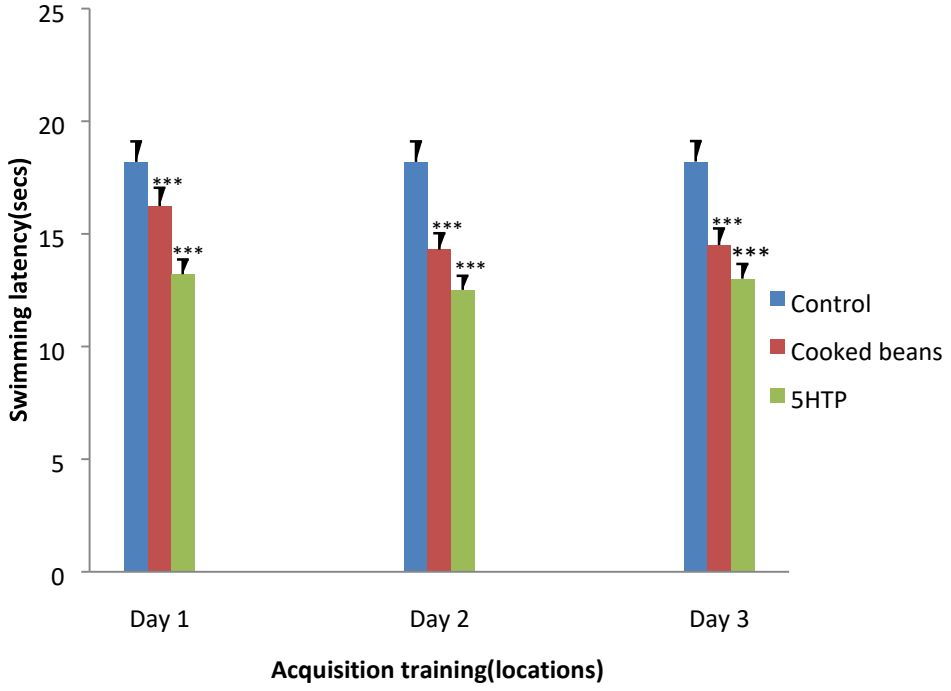

Fig 1: swimming latency during the acquisition training among the different experimental groups recorded at days 1, 2 and 3 in the Morris water maze test. Values are expressed as mean ± SEM, n = 10;*P<0.001vs.control.**

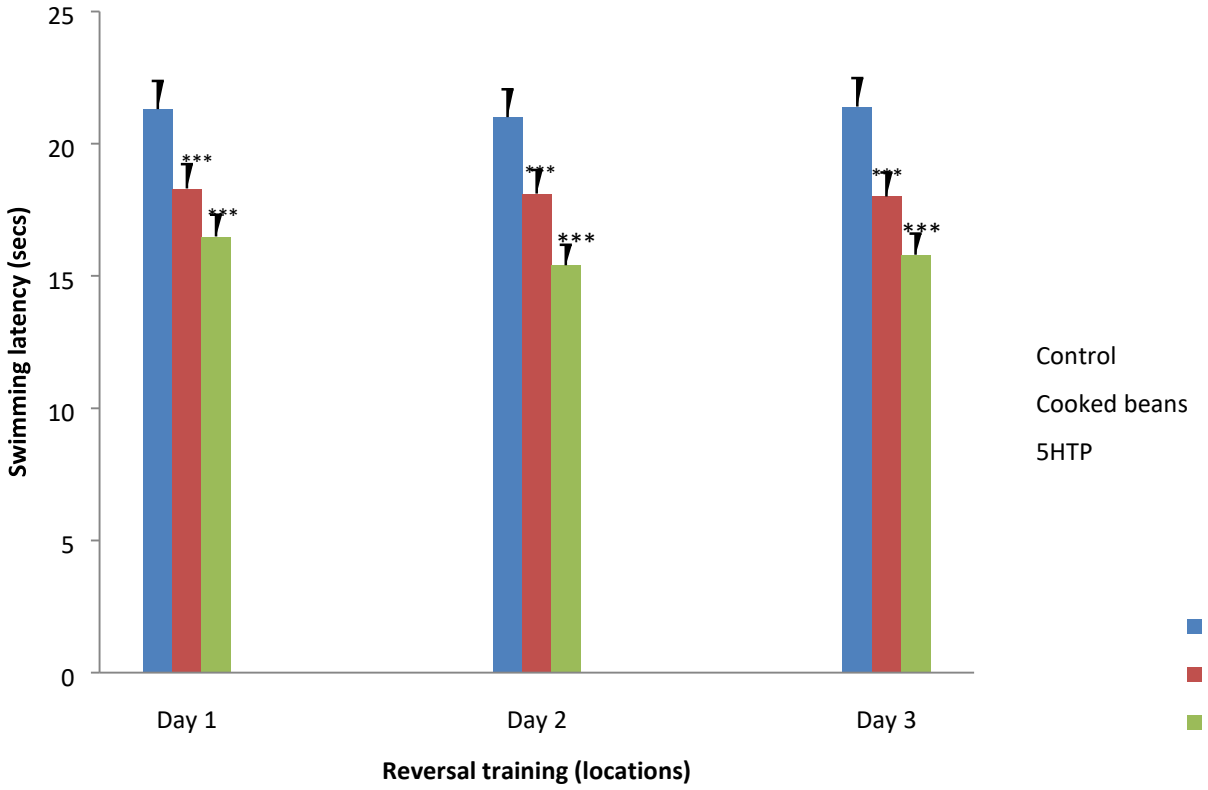

Fig 2: swimming latency during reversal training among the different experimental groups recorded at days 4, 5 and 6 in the Morris water maze test. Values are expressed as mean ± SEM, n = 10;*P<0.001vs.control.**

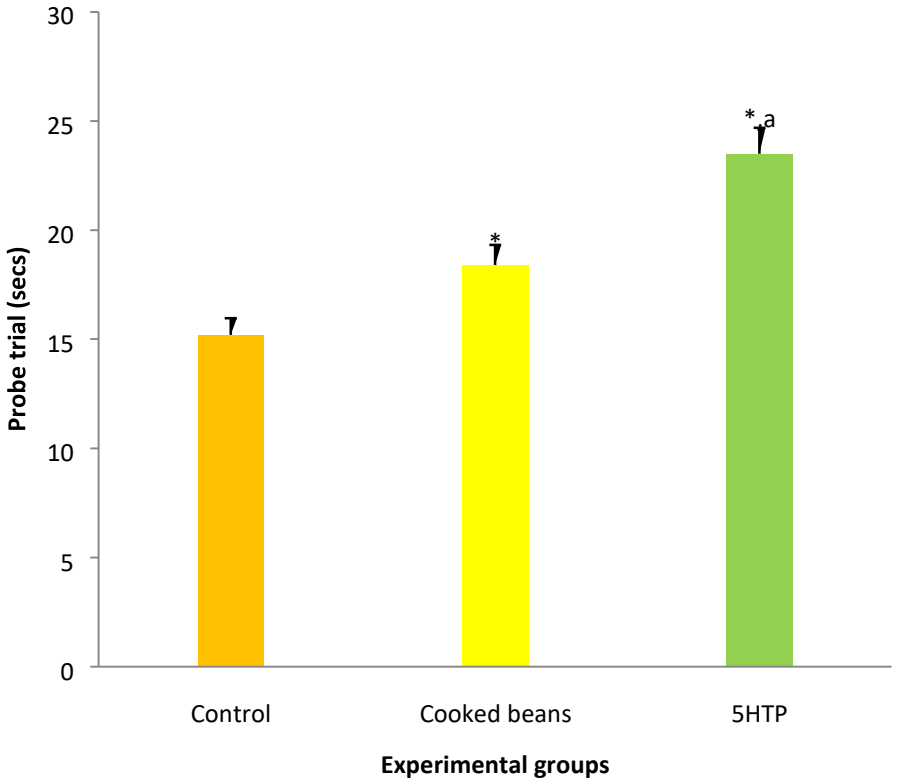

Fig 3: retention quadrant during the probe trials recorded at day 7 among the South east in the different experimental groups during the Morris water maze test. . Values are expressed as mean ± SEM, n = 10; *significantly different from control at p<0.05; a = significantly different from cooked bean at p<0.05.

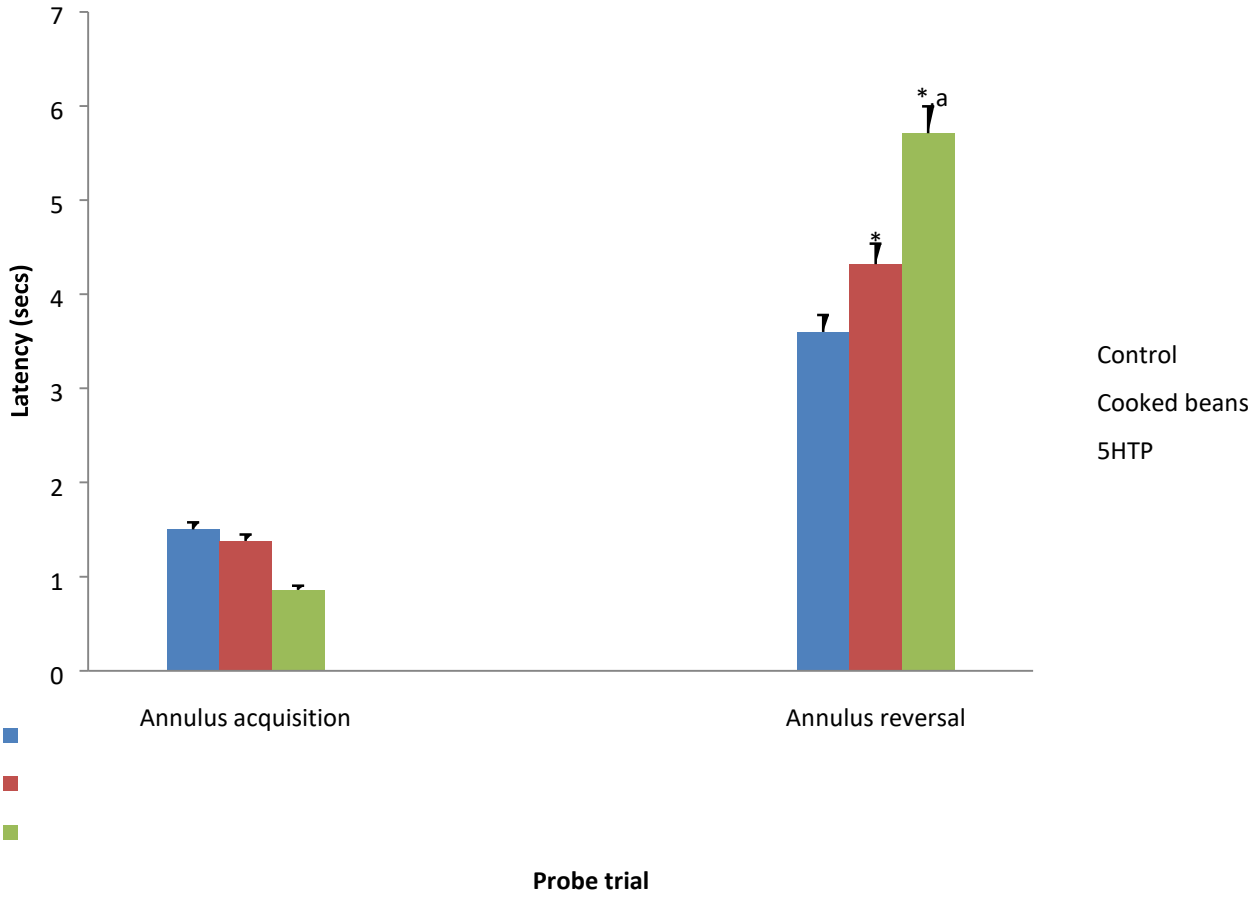

Fig 4: annulus acquisition and reversal recorded at day 7 among the different experimental groups during the Morris water maze test. . Values are expressed as mean ± SEM, n = 10; *significantly different from control at p<0.05; a = significantly different from cooked bean at p<0.05.

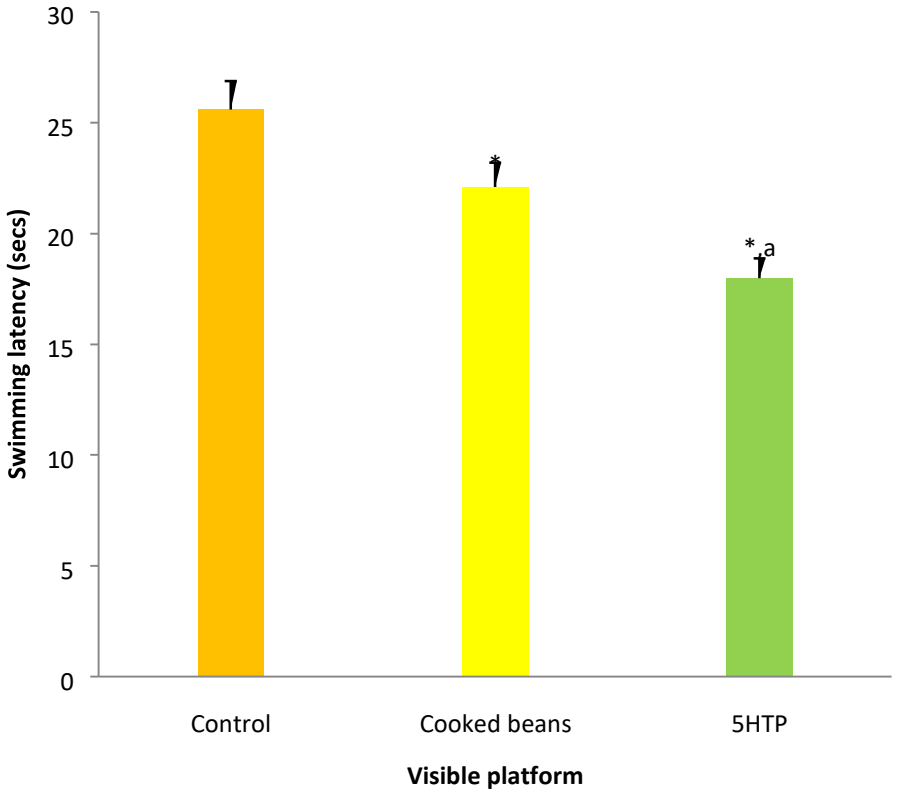

Fig 5: visible platform among the different experimental groups recorded at day 8 in the Morris water maze test. . Values are expressed as mean ± SEM, n = 10; *significantly different from control at p<0.05; a = significantly different from cooked bean at p<0.05.

3.0 DISCUSSION:

The hidden platform version of Morris water maze is a test of visuo-spatial learning and memory. This process is impaired when the hippocampus is injured (McDonald & White, 1994).The visible (cued) platform uses a unique intra-maze visual cue that is placed at the location of the escape platform whereas the visuo-spatial learning task uses extra-maze cues. The swim latency was defined as the time it took the mice to locate the hidden platform in the visuospatial learning task or cued platform task. The shorter the swim latency, the better the learning process. Mice that learn faster were able to identify the spatial location/position of the hidden platform earlier than their counter parts(within a short time).Also, the steeper the gradient of swim latencies within the three-days acquisition or reversal training, the better the learning curve, and thus learning.

Following the consumption of cooked beans and serotonin precursor diets, swim latencies for the first three days during acquisition training showed that the swim latencies of cooked beans and serotonin precursor group was significantly lower compared to the control. During reversal training, the swim latencies for the three days in the mice that consumed cooked and serotonin precursor diet were all shorter than the control group. This means that this group of mice was able to locate the hidden platform faster and so, learned faster than the control group of mice that consumed normal rodent chow. Also, the gradient of curve of the swim latencies indicates that the cooked beans and serotonin precursor fed mice learnt faster than the control.

Visuo-spatial memory was assessed during the probe trial in the Morris water maze task. During the probe trial(60 seconds exploration without hidden platform), it is expected that mice which had a good memory of the spatial location/position of the hidden platform would spend more time exploring the quadrant which had the platform during reversal training, in this case, the retention quadrant was South-East(SE) quadrant. Mice that consumed cooked beans and serotonin precursor diet spent significantly more time than the control exploring the retention quadrant. This showed that they had better memory than the control group of mice that consumed normal rodent chow.

The cued version of the Morris water maze assesses cued learning and visual integrity of the animals tested. Impairments in performance in the hidden platform model may be due to some brain lesions or drugs which may affect the motivation to escape, or sensory motor factors rather than spatial learning. This cueing procedure, in which the escape platform protrudes above the water surface, provides a control for this (Morris, 1984).Here, the swim latencies were also used for the comparisons. Shorter swim latencies in the visible platform task indicate improved cued learning. Longer swim latencies indicate poor cued learning.

The mice that consumed beans (cooked) diet and serotonin precursor diet had significantly lower swim latencies compared to the mice that consumed normal rodent chow. This means that beans consumption (cooked) improved learning process and visual integrity in mice.

Beans are rich in vitamin B6 and contain serotonin (5-HT) as well as its precursor 5Hydroxytryptophan (5-HTP) and tryptophan (Portas *et al*., 2000) in significant measures. Tryptophan hydroxylase converts tryptophan into 5-HTP which in turn is converted into serotonin (5-HT) by the enzyme aromatic amino acid decarboxylase that uses vitamin B6 as coenzyme. Serotonin is a neurotransmitter that is known to improve learning and memory as well as cognitive functions (Portas *et al*., 2000; Walther *et al.*, 2003). Furthermore, this ability of beans(cooked) diet to improve learning and memory is further enhanced by the presence of these chemical and mineral compounds such as glutamic acid(Kovalev *etal*.,1989), magnesium, potassium, phosphorus and calcium, etc., which are known to enhance memory& learning.

Our finding therefore suggests that long term consumption of beans diet enhances learning and memory. If this research can be extrapolated to humans, we recommend that the serotonergic potential of beans may be harnessed in the prevention, treatment and management of behaviour/ mental disorders as this could potentially open up a vista for improved mental health status in populations globally.

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