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Full length Research Article

The Effects of Consumption of Cooked Beans (*Phaseolus vulgaris*) and Serotonin Precursor Diets on Scopolamine-impaired Memory and Motor Co-ordination in Mice

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Summary: Alzheimer's disease (memory impairment disorder) and motor co-ordination disorders are on the increase worldwide. 5-Hydroxytryphamine (serotonin) is involved in short term memory and motor co-ordination. Whether beans that contain serotonin precursor (tryptophan) can ameliorate memory and motor co-ordination impairment has not been previously ascertained. Therefore, this research was to study the effects of consumption of cooked beans (Phaseolus vulgaris) and serotonin precursor diets on scopolamine-impaired memory and motor co-ordination in mice. Sixty mice were randomly assigned into 6 groups (10 mice per group) namely; Control, Scopolamine only, Scopolamine with 50% cooked beans diet, Scopolamine with serotonin precursor diet, 50% cooked beans diet only and serotonin precursor diet only. Preliminary studies on phytochemical analyses were done before learning/memory and motor co-ordination were also studied. Standard methods were used to study learning/memory and motor coordination. The results showed that preliminary phytochemical screening of cooked beans indicated the presence of tryptophan, flavonoids, alkaloids, and polyphenols (antioxidants). Learning was impaired in Scopolamine only group compared to control and other test groups ($p<0.05$). Memory was also impaired in scopolamine only group compared to all other experimental groups ($p<0.05$). Motor co-ordination was also impaired in scopolamine only group compared to all other groups ($p<0.05$). In conclusion, consumption of beans and serotonin precursor diets improved memory and motor coordination in scopolamine impaired memory and motor co-ordination in mice. The memory and motor co-ordination enhancement observed may be attributed to serotonin synthesized from tryptophan in bean.

Keywords*: Phaseolus vulgaris*, 5-Hydroxytryphamine, Scopolamine, serotonin precursor

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INTRODUCTION

Common bean (*Phaseolus vulgaris*) is a dicotyledonous plant belonging to family Leguminosae (Gepts, 2001). Common bean is one of the major food consumed in our communities. Beans contain: protein, carbohydrates, dietary fibres, minerals, vitamins and many phenolic compounds and is a very nutritious food from many aspects and it is not surprising that nutritionists characterize it as a nearly perfect food (Shansuddin *et al*.,1998., Vanderpoel *et al.*,1990C). It has been reported that beans have anti-carcinogenic, anti-mutagenic (Gref and Eaton (1993), anti-inflammatory, anti-diabetic, hypoglycemic, 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) (Porta *et al*., 2008). Other chemical compounds present in beans include: saponins, tannins, glycosides, flavonoids etc. Aduema (2016) reported that long term consumption of beans diet improves learning and memory in apparently normal mice.

Notable among the array of chemical constituents present in beans is serotonin which has been reported to influence neurobehavioural actions such as memory, learning, sleep, pain, feeding, sexual and emotional behaviors (Gasbarri and Pompili, 2014., Buhot *et al.*, 2000). Serotonin has been shown to act as neurotransmitter to modulate behaviour in response to changing cues, acting on both neurons and muscles to affect locomotion and learning (Cabaj *et al.*, 2017., Chase and Koelle, 2007).

Scopolamine is a drug of choice in inducing memory impairment in animals including mice. The cognitive dysfunction or memory impairment observed after this drug's usage is analogous to observations in demented patients. Scopolamine is a muscarinic receptor antagonist. It impairs long term potentiation which is responsible for long term memory (Ovsepian *et al.*, 2004). It is also used as anxiogenic agent for evaluation of anxiolytic effects of new drugs. Scopolamine has been shown to impair motor coordination in animals (Hasselmann, 2014).

Owing to the adverse effects of synthetic drugs (Deaton and Nappe, 2021). there is a search for natural remedies which are safer and effective. According to World Health Organization statistical report, 80% of the world's population presently uses traditional medicine for some aspects of primary health care including mental health (WHO, 2003). Therefore, natural products may provide a new source of beneficial neuropsychotropic drugs (Deaton and Nappe, 2021) provided they are scientifically validated and their mechanisms properly established.

Since beans contain serotonin that can potentially modulate behaviour in response to changing cues, it is, therefore, conceivable that the consumption of beans may affect learning and memory as well as motor coordination and balance. Hence, this present study investigated the effects of consumption of common beans and serotonin precursor on learning/memory and motor coordination on scopolamine impaired memory and motor coordination in CD1 mice.

MATERIALS AND METHODS

Preparation of experimental diets: Preparation of beans diet: Twenty (20) cups of beans were bought from Marian market, a local market in Calabar, Nigeria. The beans were cooked, air dried and grounded into powdered form using an electric blender. The powdered form weighed 1,560g. One kilogram of powdered cooked beans was mixed with one kilogram of normal rodent chow making 50:50(w/w) % of beans diet. The constituent was blended in a bending machine for uniform mixture.

Preparation of serotonin precursor diet: Serotonin precursor (5-Hydroxytryptophan) used for this study was obtained from Sigma Aldrich, Germany. The estimation and preparation of the powdered 5-Hydroxytryptophan content of cooked beans was according to the method of Feldman and Lee (1985) and modified by Mosienko *et al*., (2012). The serotonin precursor diet was prepared by mixing 1.15 g of the precursor in 98.85 g of the feed so that the amount of 5 HTP added was equivalent to that contained in every 100 g of cooked beans fed by the mice. An electric blender was used to blend the mixture forming the serotonin precursor diet.

Experimental animals and design: Sixty adults CD1 mice weighing between 17 – 26 g obtained from the animal house of Physiology Department, University of Calabar, Nigeria, were used for this study. They were randomly assigned into 6 groups of 10 mice each, namely; control, scopolamine only, 50% cooked beans + scopolamine, serotonin precursor diet + scopolamine, 50% cooked beans only and 5HT precursor only groups respectively. The mice in the control group were fed normal rodent chow and normal saline intraperitoneally (1ml/kg bodyweight). Group 2 mice were fed normal rodent chow and Scopolamine intraperitoneally (1mg/kg bodyweight). Group 3 mice were fed cooked beans diet and scopolamine intraperitoneally (1mg/kg bodyweight). Group 4 mice were fed serotonin precursor diet and scopolamine intraperitoneally (1mg/kg bodyweight). Group 5 mice were fed cooked beans diet and Scopolamine intraperitoneally (1mg/kg bodyweight). Group 6 mice were fed serotonin precursor diet and normal saline intraperitoneally (1mg/kg bodyweight). Scopolamine was administered once daily for the first week. In the subsequent weeks, Scopolamine was administered once every two days. The feeding and behavioural tests lasted for four weeks.

The experimental animals were kept in pathogen-free and well–ventilated housing unit at room temperature of (28 $\pm$ 2^{o}C) and humidity (85 $\pm$ 5^{o}C). The housing rooms were illuminated on a 12-hour light-dark cycle. The animals were allowed access to water and food ad libitum. Approval for the use of the animals was obtained from the College Ethical Committee of University of Calabar, Nigeria on the use of experimental animals and it was in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guide lines (EEC Directive of 1986; 86/609/EEC).

Behavioural Protocols

Morris water maze for learning and memory: A Morris water maze modified for mice was used to study learning and memory in this study (Parlor *et al*., 1996), which is smaller than the maze developed for rats. The water maze is constructed out of a circular polypropylene pool (Canadian tire "Pelican" pool) that measures 110cm in diameter and 20cm in depth. The pool was filled to a depth of 14-cm (0.5-cm over the platform) with room-temperature tap water, which was made opaque with the addition of 100mL of non-toxic white liquid tempura paint. The water was left to sit overnight in order to attain room temperature (22 ± 1°C).

The pool was divided into four quadrants: Northwest, Northeast, Southwest and Southeast. Boundaries of these quadrants were marked on the edges of the pool with masking tape and labelled: North, South, East and West. A Plexiglas cylinder (13.75 cm x 9 cm diameter) was used as the escape platform in the maze. The cylinder was been filled with cement to weigh it down in the pool. The platform had a removable red and yellow striped top (3 cm x 9 cm in diameter) with a colorful flag erected in the centre. For visible platform tests the level of the water in the pool was adjusted to 0.5-cm below the surface of the striped top, thus creating a visible escape platform, or to 0.5-cm above the white cylinder (with the striped top removed), creating a hidden escape platform.

The pool was located in a room measuring 5.2 x 2.4 m. Several posters were placed on the walls of the room to act as visual cues. There was also furniture in the room (sink, table, chairs) that provides visual cues. During testing, the room was dimly lit with diffuse white light (30 lux). The performance of the animals in the water maze was recorded using a video camera-based computer tracking system (Water maze, Actimetrics) on an IBM PC computer, with the camera fixed to the ceiling 2.1m above the pool.

In our paradigm, testing in the water maze lasted for 8 days: days 1-3 was acquisition training, 4-6 reversal training, day 7 probe trial and day 8 is visible- platform days respectively. Acquisition and reversal training were with the hidden platform (water is 0.5-cm above platform). During reversal, the platform was moved to the opposite side of the maze. During the probe trial, there was no escape platform so that visuo-spatial memory can be assessed. On the

visible-platform day the platform was moved to another quadrant of the pool and the visible top is added to the platform. This assesses basic visual ability and motivation to locate the platform. Each day, the mouse was removed from its home cage and was placed in a clean holding cage without woodchip bedding. Paper towel was torn into strips and placed in the bottom of the holding cages to allow the mice to dry more quickly. This paper towels were replaced when it became wet. Mice were run in squads of 4-6 with 5-minutes between each trial (inter-trial interval) for each mouse (Iivonen *et al.,* 2003).

During acquisition training, the platform was placed in the centre of the Northeast quadrant. Each mouse received 4 trials per day. In each trial, the mouse was given a maximum of 60-sec to locate the escape platform. The starting positions of the mice were predetermined using a Latin square design, which prevents the repetition of starting location sequences on back-to-back test days. Possible start positions were at the boundaries of the quadrants (e.g, West, North, East or South). For each trial, each mouse was removed from its holding cage using a small, clean 500-mL plastic container to minimize handling stress. The animal is then placed into the water at the appropriate start position. The mouse was then permitted to explore the pool and to search for the hidden escape platform for 60-sec. When the animal located the platform, the timer was stopped (manually) and the mouse was allowed to stay on the platform. Once on the platform, the mice were permitted to view the extra-maze environment for 10-sec. If the mouse does not find the platform during the allotted time, the animal was guided onto the platform using the plastic container. The next mouse is then placed in the pool and the same procedure followed. Each animal completes 4 trials per day over 3 days, for 12 trials of acquisition training, each trial from a different one of the 4 start locations.

Reversal training began on day 4. The invisible platform was moved to the opposite quadrant (Southwest quadrant), and mice are again assigned to appropriate start positions. The same procedures as in acquisition training were carried out during reversal training. Each of the animals completes 4 trials per day for 3 days for a total of 12 trials of reversal training. A probe trial was conducted on day 7 to assess visuo-spatial memory. At this time, there was no escape platform in the maze. Each mouse was placed in the pool from one of the four possible start positions and allowed to explore the pool for 60-sec, during which the time spent in each quadrant of the maze is recorded. When the 60-sec is complete the mouse is scooped up using the container and placed in a holding cage to dry before being returned to its home cage.

The visible platform task was conducted on day 8. The visible platform was placed in a new location within the Northwest quadrant of the pool. The same procedures as in acquisition and reversal training are carried out and mice complete 4 trials. During acquisition, reversal and visual training, the following behaviours were measured: swim latency (time to find and mount the escape platform), swim distance, proximity to the platform.

During the probe trial, the measures recorded were: frequency of entries into each quadrant (Northeast, Northwest, Southeast and Southwest), duration of time spent in each quadrant, the number of times the mouse crosses the location of the platform during reversal training (annulus reversal crossing), the number of times the mouse crosses the location of the platform during acquisition training (annulus acquisition crossing), the duration and frequency of thigmotaxic behaviour (9 cm corridor width) and proximity to the platform location.

The novel object recognition task for memory: The novel object recognition task (NORT) was originally designed for rats as a test of declarative memory, after it was discovered that rats will spend more time investigating a new object than a familiar one (Ennaceur and Delacour, 1988). It has since been validated as a test of recognition memory in mice (Brown *et al*., 1999; Podhorna and Brown, 2002; and Sik *et al*., 2003). This test which has many variants is based on a spontaneous behaviour in animals an unconditioned preference for novel objects. The modified method of Brown *et al.* (2002) was used in this study.

Prior to testing all mice were allowed to familiarize with the apparatus for 5 minutes before the test commences. Mice were carried to the test room in their home cages. They were moved from their home cages to the testing apparatus and back using a small container. After each 5 minutes trial, the mice were returned to their cages and the apparatus was cleaned with 70 % ethyl alcohol and permitted to dry between trials.

The behaviours scored during the Open Field (NORT) include: Approaches to Each Object: directing the nose to the object at a distance of < 1 cm and/or touching it with the nose and Time Spent with Each Object: sniffing or climbing the object.

Beam walking for determination of motor coordination: motor coordination was assessed using the beam walking test. The beam walking apparatus was used to test motor coordination and balance. The beam walking test is more sensitive than the mouse rotarod in determining motor coordination deficits (Stanley *et al.,*2005). The beam has a length of 100 cm, a width of 2 cm and is elevated to a height of 40 cm. The beam is marked at 5 cm and 1 cm intervals. It is composed of wood and is coated with black paint. The animals were carried to the test room in their home cage. The mouse was removed from its home cage and placed at one end of the balance beam. After the mouse had secured its grip on the beam, the trial began. The maximum length of the trial is two minutes. The mouse was tested under white light, during the dark phase. The beam was cleaned with 70% ethanol and permitted to dry between each trial. Behaviour scored were**:** Distance travelled, Foot Slips, Number of turns and Latency to fall.

Statistical Analysis Data obtained were presented as mean ± SEM. Experimental data were analyzed using analysis of variance (ANOVA) followed by a post hoc test (Least Square Difference (LSD) test) to determine significant difference between means. Th e analysis was done with an SPSS 18 statistical package. The mean values were considered significant at $p<0.05$.

RESULTS

Phytochemical screening: Preliminary results of the phytochemical analysis of beans showed the presence of large quantities of flavonoids, alkaloids and polyphenols that are antioxidants. The result is presented in Table 1.

Behaviours scored in Morris water maze

Comparison of swim latency during the acquisition training: Figure 1 shows the swim latencies during the acquisition training in the Morris water maze (Days 1, 2 and 3) between the control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans and 5HT precursor diet. The swim latencies in day 1 were 59.67 ± 0.223, 57.85 ± 1.365, 57.14 ± 1.825, 56.31 ± 1.621, 57.90 ± 0.903 and 58.55 ± 1.270 seconds for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans and 5HT precursor diet groups respectively. The result showed that, there was no significant difference between the experimental groups compared to control in day 1. However, in day 2 the swim latency of 50% cooked beans (38.26 ± 3.534 sec) was significantly shorter compared to Control and other experimental groups ($p<0.05$). In acquisition day 3 the swim latency of the scopolamine only group was significantly longer (47.41 ± 3.088 sec) compared to control and other experimental groups.

Comparison of swim latency during the reversal training: Figure 2 represents the swim latency curves during the reversal training in the Morris water maze (Day 4, 5 and 6) between the control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet. The result shown that, in reversal training day 1 the swim latency (39.70 ± 3.822) of the 50% cooked beans diet was significantly shorter compared to control and other experimental groups ($p<0.05$). Similar trend was observed in reversal training day 2. In reversal training day 3 the results for the swim latencies were 32.04 ± 2.895, 49.22 ± 2.111, 16.17 ± 2.902, 19.06 ± 3.482, 15.78 ± 2.241 and 18.29 ± 3.211 seconds for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively. The scopolamine only group was significantly longer compared to control and other experimental groups ($p<0.05$). The result also showed that, the swim latencies of the experimental groups fed with serotonin precursor and cooked beans diets were significantly shorter latency compared to control ($p<0.05$)

Table 1:
Phytochemical analysis of cooked beans

S/N	Chemical Compound	Component Value
1	Alkaloids	+ +
2	Phlobatannin	+
3	Saponins	+ +
4	Flavonoids	+ + +
5	Tannins	+ +
6	Hydroxymethyl Anthraquinone	_
7	Cardiac Glycoside	+
8	Polyphenol	+ + +
9	Reducing Sugars	+ +
10	Anthraquinone	_

Key:
+ + + = *highly present*
+ + = *moderately present*
+ = *slightly present*
- = *absent*

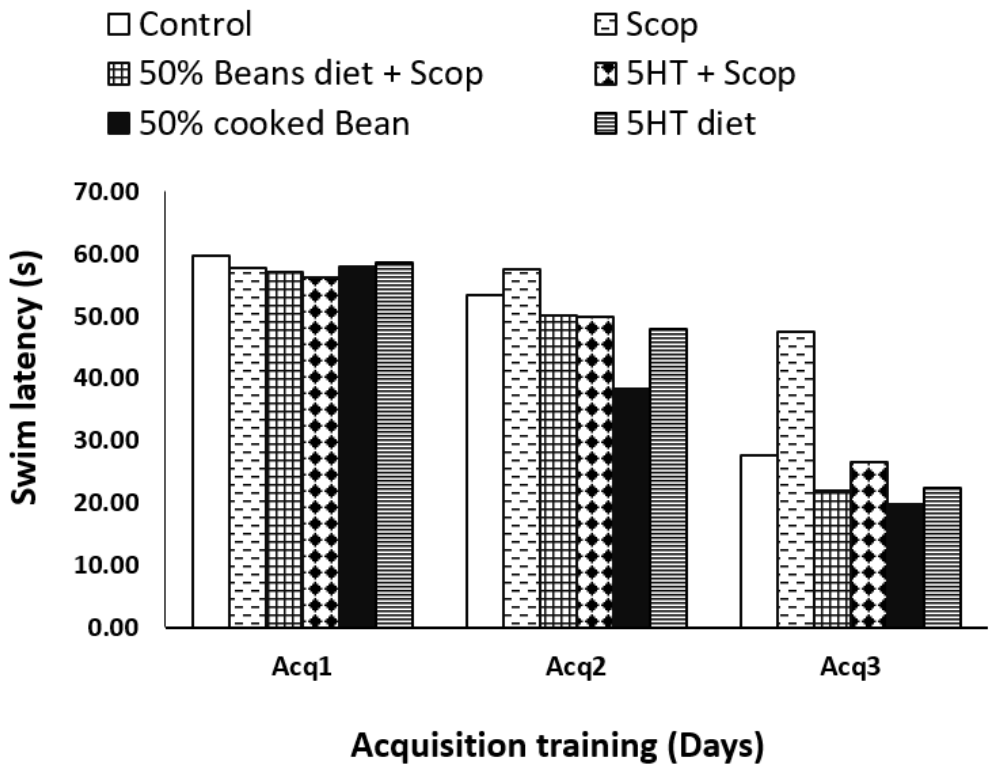

Figure 1:
Comparison of acquisition training of control and test groups in Morris water maze.
Value are expressed as mean ± SEM, n = 10.

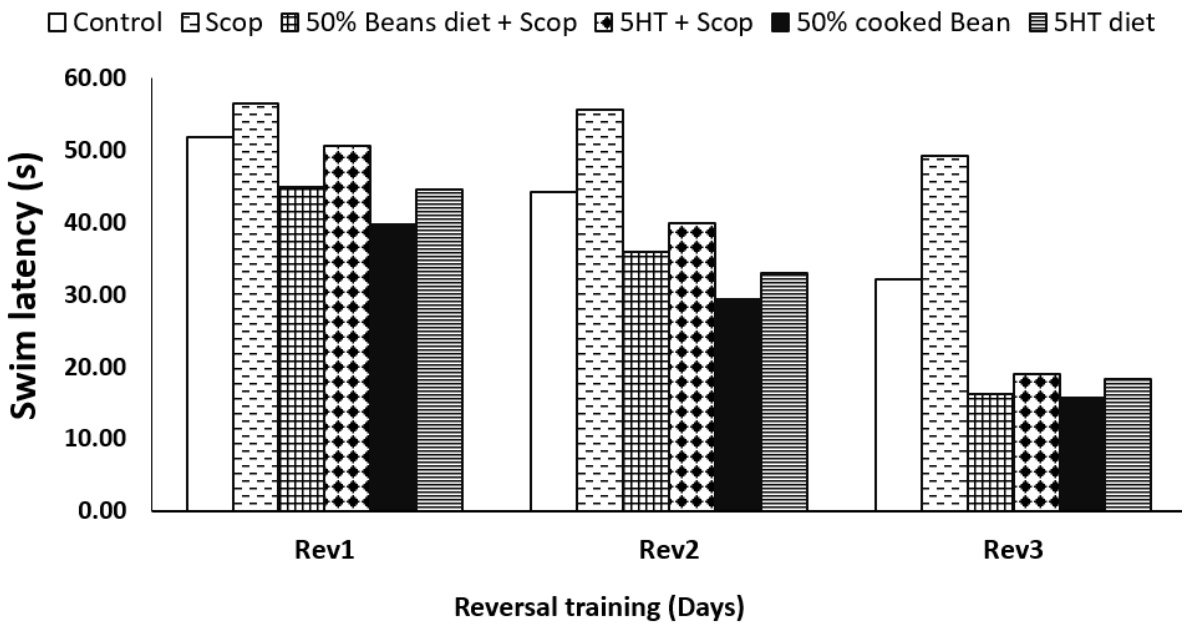

Figure 2:
Comparison of reversal training of control and test groups in Morris water maze.

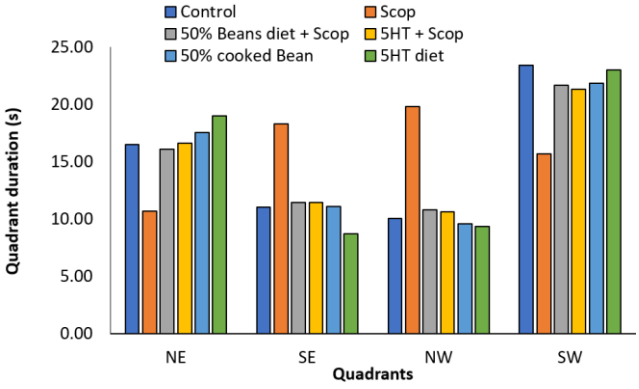

Figure 3:
Comparison of quadrant duration during probe trial test of control and test groups in Morris water maze.

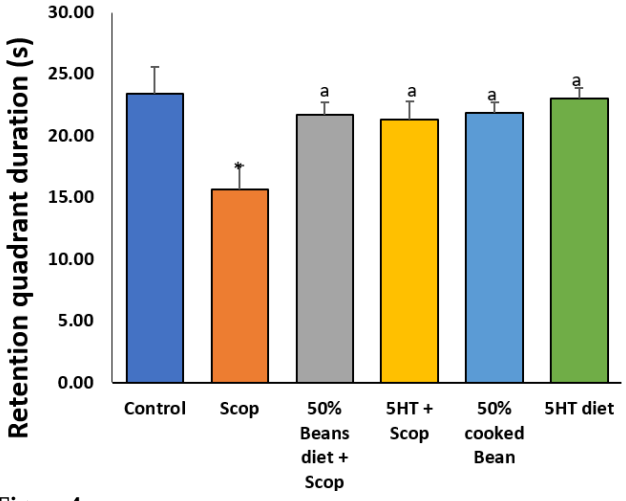

Figure 4:
Comparison of Retention quadrant duration of control and test groups in Morris water maze.
Value are expressed as mean ± SEM, n = 10.
** = p<0.05 vs control; a = p<0.05 vs scopolamine*

Comparison of quadrant duration: Figure 3 represents comparison of the quadrant duration during probe trial test in the Morris water maze. The mean duration in each quadrant were: northeast (16.51 ± 1.341, 10.69 ± 1.035, 16.08 ± 0.653, 16.61 ± 0.679, 17.53 ± 0.667 and 18.96 ± 0.749 for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively), southeast (11.042 ± 1.598, 18.30 ± 1.429, 11.43 ± 1.004, 11.44 ± 0.858, 11.08 ± 0.637 and 8.69 ± 1.019 for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively), northwest (10.048 ± 1.093, 19.80 ± 2.406, 10.81 ± 0.659, 10.62 ± 0.932, 9.55 ± 0.845 and 9.32 ± 0.817 for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively) and southwest (23.40 ± 2.171, 15.68 ± 2.389, 21.67 ± 1.061, 21.32 ± 1.493, 21.82 ± 0.859 and 23. 01 ± 0.825 for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively). The result showed that, the control, cooked beans and serotonin precursor diets groups expressed significant preference to the northeast and southwest quadrants compared to the group treated with scopolamine only ($p<0.05$).

Duration of quadrant retention: Figure 4 represents comparison of the quadrant duration retention during the probe trial in the Morris water maze test between the Control, scopolamine only, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet having durations: 23.40 ± 2.17, 15.68 ± 1.88, 21.67 ± 1.06, 21.32 ± 1.49, 21.3 ± 0.85 and 23.02 ± 0.82 seconds respectively. The result shown significantly reduced quadrant retention compared to control and other experimental groups ($p<0.05$). There was no significant difference between other experimental groups compared to control.

Annulus acquisition frequency: The annulus acquisition crossing during the probe trial task frequencies were 2.26 ± 0.176, 1.20 ± 0.313, 2.20 ± 0.133, 1.89 ± 0.209, 2.56 ± 0.156 and 2.44 ± 0.193 for Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively. The result shows that the annulus acquisition crossing for the scopolamine only group was significantly reduced compared to control and other experimental groups ($p<0.05$). There was no significant difference between experimental groups compared to control. The result is illustrated in figure 5.

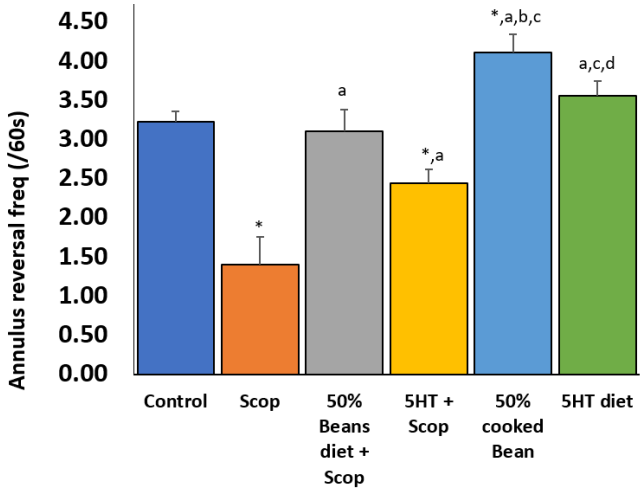

Figure 5:
Comparison of annulus reversal frequency of control and test groups in Morris water maze.
Value are expressed as mean ± SEM, n = 10.
** = p<0.05 vs control; a = p<0.05 vs scopolamine*
b = p<0.05 vs 50% cooked beans + scopolamine
c = p<0.05 vs 5HT precursor + scopolamine
d = p<0.05 vs 50% cooked beans

Annulus reversal crossing frequency: The annulus reversal crossing during the probe trial task frequencies were 3.22 ± 0.133, 1.40 ± 0.30, 3.10 ± 0.276, 2.44 ± 0.175, 4.11 ± 0.230 and 3.56 ± 0.193 for Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively. The result shown that, the annulus reversal crossing for the scopolamine only group was significantly reduced compared to control and other experimental groups ($p<0.05$). The 50% cooked beans only group also shown a significant increase in reversal crossing compared to control and other experimental groups ($p<0.05$). The result is illustrated in Figure 6.

Comparison of swim latencies on the visible platform test: The swim latencies during visible platform test for Control, scopolamine only, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet, were 15.73 ± 2.461, 37.76 ± 2.360, 10.95 ± 1.755, 14.83 ± 1.566, 8.09 ± 0.794 and 12.64 ± 0.719 seconds respectively. From the result, the swim latency for the scopolamine only group was significantly longer compared to control and other experimental groups ($p<0.05$). The 50% cooked beans only group, showed significantly shortest latency during the visible test compared to control and other experimental groups ($p<0.05$). see Figure 6 below.

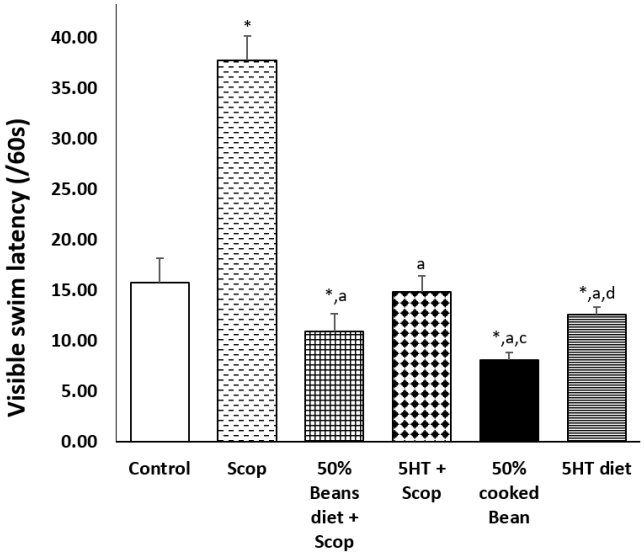

Figure 6:
Comparison of visible swim latency of control and test groups in Morris water maze.
Value are expressed as mean ± SEM, n = 10.
** = p<0.05 vs control; a = p<0.05 vs scopolamine*
b = p<0.05 vs 50% cooked beans + scopolamine
c = p<0.05 vs 5HT precursor + scopolamine
d = p<0.05 vs 50% cooked beans

Behaviours scored in novel object recognition test

Comparison of the habituation index for short term memory: Figure 7 represents the mean habituation index for short term memory for the Control, scopolamine only, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet are 49.14 ± 3.05, -4.30 ± 2.40, 52.98 ± 4.57, 48.31 ± 4.03, 57.61 ± 3.13 and 54.44 ± 4.14 respectively. The habituation index for short term memory of the Scopolamine only group was significantly lower compared to control and other experimental groups ($p<0.05$). There was no significant difference between the other experimental groups compared to control.

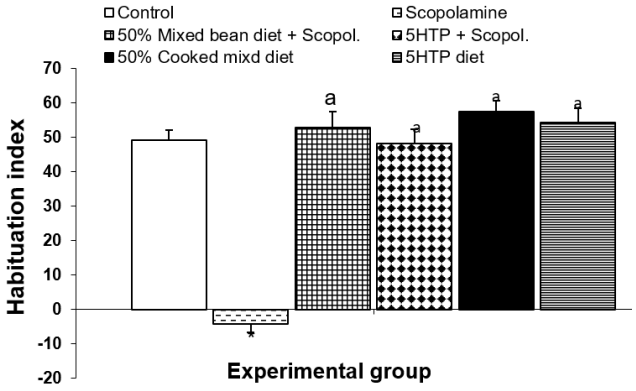

Figure 7:
Comparison of habituation index for short term memory during the NORT in control and test groups
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control; a = p<0.05 vs scopolamine

Comparison of the habituation index for long term memory: Figure 8 represents the mean habituation index for long term memory for the Control, scopolamine only, 50% cooked beans +scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet (7.64 ± 1.86, -8.17 ± 2.91, 5.27 ± 3.82, -1.94 ± 1.19, 13.00 ± 1.09 and 11.89 ± 2.07) respectively. The habituation index for long term memory of the Scopolamine only group was significantly lower compared to control and other experimental groups ($p<0.05$). The mean values of the groups fed with cooked beans only and serotonin precursor diet only were significantly higher compared to control ($p<0.05$).

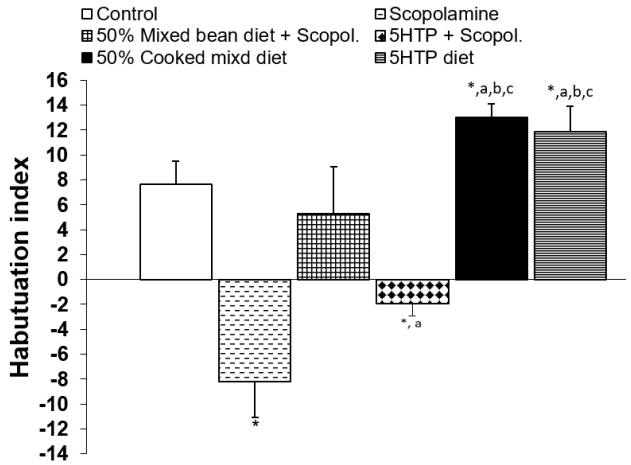

Figure 8:
Comparison of Habituation index for long term memory during the NORT in control, 50% cooked beans, 5HT precursor and scopolamine treated groups
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control; a = p<0.05 vs scopolamine
b =50% cooked mixed diet; c = p<0.05 vs 50% mixed bean diet+Scopol

Comparison of the discrimination index for short term memory: Figure 9 represents the mean discrimination index for short term memory for the Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet (0.51 ± 0.06, -0.18 ± 0.03, 069 ± 0.15, 0.52 ± 0.01, 0.64 ± 0.14 and 0.52 ± 0.12) respectively. The mean discrimination index for short term memory of the Scopolamine only group was significantly lower compared to the control and other experimental groups ($p<0.05$).

Comparison of the discrimination index for long term memory: The mean discrimination index for long term memory for the Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet (0.12 ± 0.03, -0.13 ± 0.03, 0.03 ± 0.02, -0.08 ± 0.02, 0.14 ± 0.01 and 0.10 ± 0.01) respectively. The mean discrimination index for long term memory of the Scopolamine only group was significantly lower compared to the control and other experimental groups ($p<0.05$). The group fed with 50% cooked beans only had longer discrimination index compared to control and other experimental groups ($p<0.05$). The group treated with 50% cooked beans diet + scopolamine and serotonin precursor diets + scopolamine had significantly decreased discrimination index compared to control ($p<0.05$).

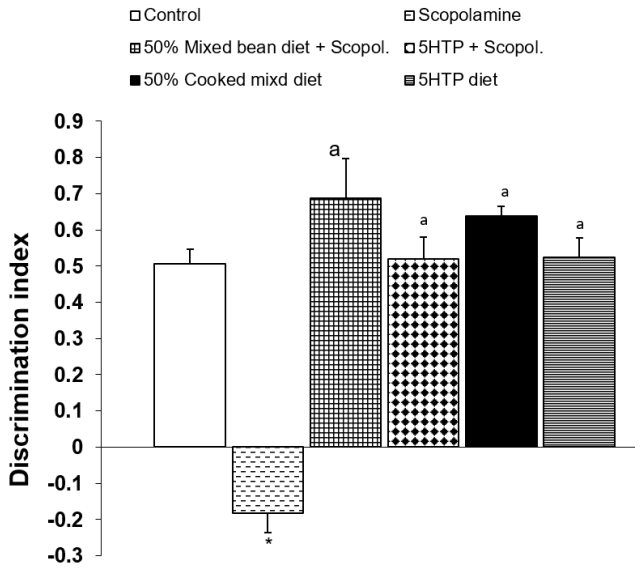

Figure. 9:
Comparison of discriminative index for short term memory during the NORT in control and test groups
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control; a = p<0.05 vs scopolamine

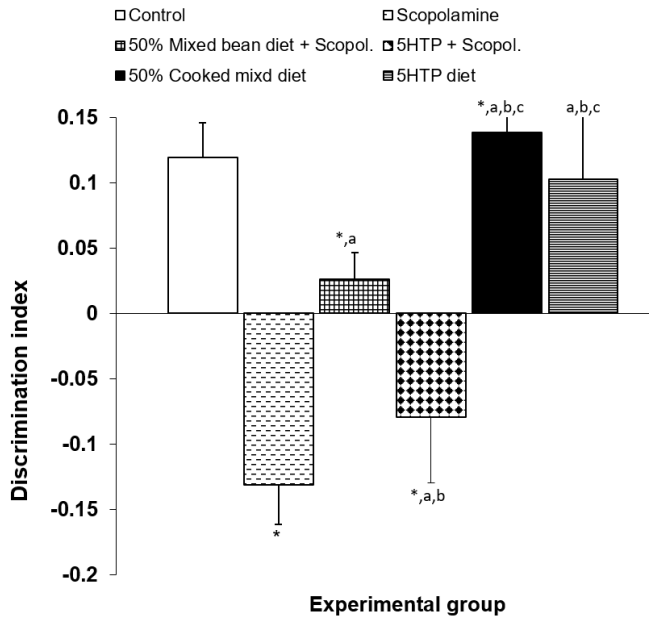

Figure. 10:
Comparison of Discriminative index for long term memory during the NORT in control and test groups
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control; a = p<0.05 vs scopolamine
b = 50% cooked mixed diet
c = p<0.05 vs 50% mixed bean diet + Scopolamine

Behaviours scored in beam walking

Line crosses: Figure 11 represent the comparison of mean line crosses for control, scopolamine only, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet in beam walking (429.89 ± 19.57, 232.20 ± 20.36, 476.80 ± 30.76, 387.78 ±

29.18, 471.11 ± 14.55 and 393.33 ± 18.23) respectively. The line crosses of the scopolamine only administered group was significantly decreased compared to control and other experimental groups ($p<0.05$). The result also showed that, groups fed with cooked beans diet shown a significant increased line crosses compared to control and serotonin precursor diet fed groups ($p<0.05$). There was no significant difference between the serotonin precursor diet groups compared to control.

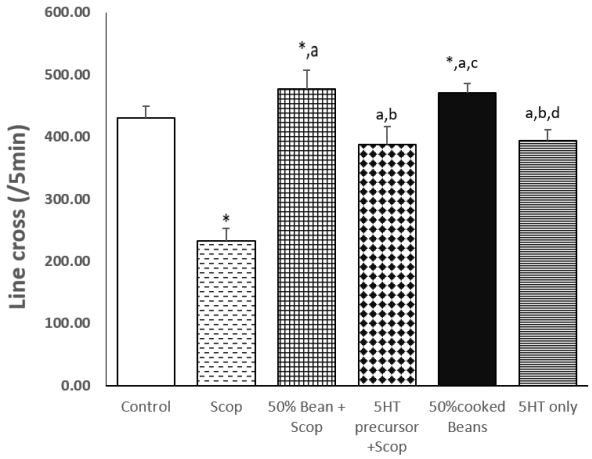

Figure 11:
Comparison line cross of control and test groups in walking beam
Value are expressed as mean ± SEM, n = 10.
** = p<0.05 vs control; a = p<0.05 vs scopolamine*
b = p<0.05 vs 50% cooked beans + scopolamine
c = p<0.05 vs 5HT precursor + scopolamine
d = p<0.05 vs 50% cooked beans

Foot Slips: The mean frequencies of foot slips of the different experimental groups was recorded as follows: 1.33 ± 0.47, 13.00 ± 0.98, 2.30 ± 0.50, 4.22 ± 0.32, 1.00 ± 0.37 and 1.67.050 for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet respectively. The result indicated that, the number of foot slips in the group treated with scopolamine only was significantly increased compared to control and other treatment groups ($p<0.05$). There was no significant difference between 50% cooked beans + scopolamine, 50% cooked beans, and 5HT precursor diet compared to control. The foot slips in 5HT precursor diet + scopolamine group was significantly increased compared to control ($p<0.05$; Figure 12).

Reversals: Figure 13 below represent the comparison of mean reversals of control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet in beam walking were 4.89 ± 0.39, 7.70 ± 0.42, 4.70 ± 0.63, 4.67 ± 0.50, 4.33 ± 0.17 and 4.33 ± 0.24 respectively. The results showed that there was a significant increase in mean number of reversals made by the scopolamine group compared to control and other treatment groups ($p<0.05$). there was no significant difference between the treatment groups compared to control.

DISCUSSION

The effects of consumption of cooked beans (*Phaseolus vulgaris*) and serotonin precursor diets on scopolamine-impaired memory and motor co-ordination in mice were studied. The effects of serotonin precursor (5-Hydroxytryptophan) diet were also compared with cooked beans diet because 5-Hydroxytryptophan is one of the constituents of beans. Preliminary phytochemical screening of beans was done before parameters for learning/memory and motor coordination were also studied. The Morris water maze and Novel object recognition tests were used to estimate learning and memory, and beam walking test was used to study motor co-ordination and balance.

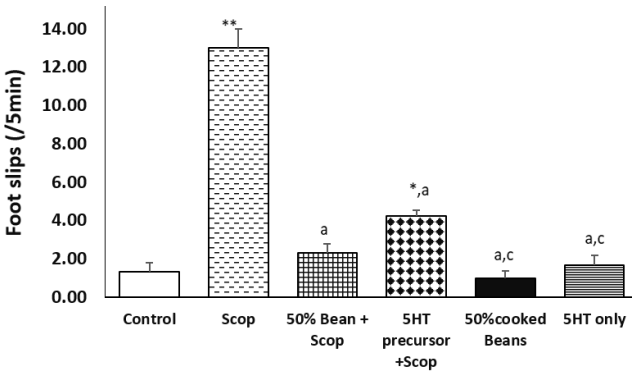

Figure 12:
Comparison of foot slips of control and test groups in walking beam.
Values are expressed as mean ± SEM, n = 10.
*** = p<0.001 vs control; *= p<0.05 vs control*
a=p<0.001 vs scopolamine
c=p<0.05 vs 5HT precursor + scopolamine

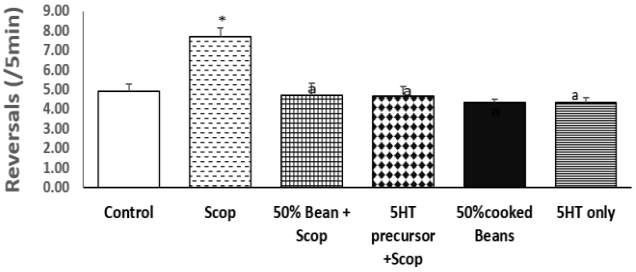

Figure 13:
Comparison of reversals of control and test groups in walking beam
Values are expressed as mean ± SEM, n = 10.
*** = p<0.001 vs control; *= p<0.05 vs control*
a=p<0.001 vs scopolamine
c=p<0.05 vs 5HT precursor + scopolamine

Preliminary results of the phytochemical analysis of beans showed the presence of large quantities of flavonoids, alkaloids and polyphenols that are antioxidants. Flavonoids have been found to improve memory and motor coordination in mice. The results of this study show that the groups fed cooked beans and serotonin precursor diets shown improved learning and memory as well as motor co-ordination compared to scopolamine only treated group. This observation is consistent with the earlier study carried out by Samira *et al.,* (2015) who reported that, antioxidants improved motor co-ordination in mice with impaired memory and motor deficit.

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 all the groups fed cooked beans and serotonin

precursor diets were significantly lower compared to control and the scopolamine only treated group.

The same trend was observed during the reversal training days. This means that these groups of mice were able to locate the hidden escape platform faster and so, learned faster compared to control and scopolamine only treated groups that consumed normal rodent chow. The scopolamine only group had poorer learning curve.

Visuo-spatial memory was assessed during the probe trial in the Morris water maze task. Mice fed cooked beans and serotonin precursor diets and the control group spent significantly more time exploring the retention quadrants compared to the group treated with scopolamine only. This showed that they had improved memory compared to scopolamine only treated group of mice.

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 sensorymotor factors rather than spatial learning. 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 fed both cooked beans as well as serotonin precursor diets had significantly shorter swim latencies compared to the control and scopolamine only treated group. This means that consumption of cooked beans and serotonin precursor diets improved learning process and visual integrity in mice.

Beans are rich in vitamin B6 and contain 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 acid decarboxylase that uses vitamin B6 as co-enzyme. 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, the property of beans diet improving learning and memory is further enhanced by the presence of mineral compounds such as glutamic acid (Yehuda *et al*., 1996), magnesium, potassium, phosphorus and calcium, etc. which are known to enhance memory and learning.

From the results obtained, the habituation index for short term memory for the Scopolamine only treated group was lower compared to other experimental groups, whereas the values from the groups fed cooked beans and serotonin precursor diets groups appeared higher. Decrease in habituation depicts deficit in associative learning (Bolivar, 2010). The Scopolamine only treated group was also observed to have decreased long term habituation index, thus confirming their memory deficits. The long term habituation index for the animals fed cooked beans and serotonin precursor diet appeared to be higher than that of the Scopolamine only treated group. This increased habituation could be interpreted as improvement in the memory of these animals (Blackford *et al.*, 2013).

short term memory capabilities of group of mice fed cooked be beans diet was the highest as shown by the significant increase in intrasession habituation. However, the animals fed serotonin precursor diet had improved long term memory as shown by the significant increase in intersession habituation index.

From the results obtained, the discrimination index for short term memory of the Scopolamine only treated group was significantly lower compared to control and other experimental groups, while the value for other treated test groups showed no significant difference compared to control. However, the result of discrimination index for long term memory showed that the values for the animals fed cooked beans and serotonin precursor diets were observed to be more positive compared to the Scopolamine only treated group, with that of the 50% cooked beans diet group showing the highest. The increased positivity of these groups indicates more time spent exploring the novel object and thus implied improvement in recognition memory. These results depict that, cooked beans diet improved both short- and long-term memory of the mice whereas, serotonin precursor diet improves short term memory. The observed memory enhancement following cooked beans diet consumption may be due to the involvement of serotonin present in cooked beans diet.

The results obtained from beam walking showed that, the groups of mice fed cooked beans and serotonin precursor diets showed better motor coordination compared to scopolamine only treated group. The scopolamine only treated group had a poor motor co-ordination compared to control. It is possible that scopolamine suppressed motor centers in the brain as indicated in the results showing decreased line crossings, a higher number of foot slips and greater number of reversals. The result also shows that the group of mice fed cooked beans diets had higher significant motor co-ordination compared to control and other experimental groups. This is because, decreased frequency of food slips and increase line crosses indicate a higher level of maneuverability in the beam, thus indicating better motor learning ability.

The results of this study shows that the groups of mice fed with cooked beans as well as serotonin precursor diets had improved motor co-ordination compared to control and scopolamine only treated group. This observation is consistent with the earlier study carried out by Samira *et al.*, (2015). Who reported that, antioxidants improved motor co-ordination in mice with impaired memory and motor deficit. The effects of consumption of cooked beans (*Phaseolus vulgaris*) and serotonin precursor diets on scopolamine-impaired memory and motor co-ordination in mice were studied. Preliminary studies on phytochemical analysis of the beans were studied before learning and memory as well as motor coordination. Preliminary results of the phytochemical analysis of beans showed the presence of large quantities of flavonoids, alkaloids and polyphenols that are antioxidants. Learning was impaired in animals treated with scopolamine only compared with control and other test groups ($p<0.05$). Memory was also impaired in the group of animals treated with scopolamine only compared to control and other experimental groups.

Motor coordination was impaired in animals treated with scopolamine only compared to control and other experimental groups($p<0.05$). Consumption of beans as well as serotonin precursor diets improved learning and memory as well as motor coordination.

In conclusion, consumption of cooked beans and serotonin precursor diets improved learning and memory as well as motor coordination. The learning and memory enhancement observed may be attributed to antioxidants and

serotonin precursor (tryptophan) present in cooked beans diet and the motor coordination enhancement may also be due to antioxidants present also in cooked beans.

REFERENCES

Wader, J. K. P., Telek, L., VozariHampem and Sami, H.S. [1998]. Antinutritional factors in Anasazi and other Pinto beans [Phaseolusvulgaris]. *Plant Foods for Human Nutrition*, 51[2]: 85-98.

Shansuddin, A. M and Elsayed A. [1998]. Suppression of large intestinal cancer in F344 rats by inositol Hexaphosphate *Carcinogenesis*, 9[4]:577-580

Vanderpoel, A.F.B, Liener L.E., Mollee, P.W and Huisman, J (1990C). variation among species of animals in response to the feeding of heat processed beans (Phsaseolus vulganis.2) Growth and organ weights of chickens and rats and digestibility of rats. *Livestock production sciences*, 25(1-20: 137-150.

Gref, E. and Eaton, J.W (1993). Suppression of Caloric Cancer by Dietary phyto Acid. International Journal of Nutrition and Cancer, 19 (1): 11-19

Bennick, E., Maurice, O. and Elizabeth R. [2008]. Beans & Health: A Comprehensive Review, *Frazee*, MN.

Porta, H., Figueroa-Balderas, R. E., & Rocha-Sosa, M. (2008). Wounding and pathogen infection induce a chloroplast-targeted lipoxygenase in the common bean (Phaseolus vulgaris L.). *Planta*, 227(2), 363-373.

Aduema, W. (2016). Effects Of Long-Term Administration Of Cooked Beans (Vigna Unguiculata) Diet On Learning And Memory In Mice. *International Journal Of Food Sciences*, 1(1), 1-15.

Patras, A., Tiwari, B. K., & Brunton, N. P. (2011). Influence of blanching and low temperature preservation strategies on antioxidant activity and phytochemical content of carrots, green beans and broccoli. LWT-Food Science and Technology, 44(1), 299-306.

Chase DL, Koelle MR. (2007). Biogenic amine neurotransmitters in C. Elegans. *WormBook*. Feb 20:1-15. doi: 10.1895/wormbook.1.132.1.

Young S.N and Teff, K. L (1989) Tryptophan availability, 5-HTP synthesis and 5-HT function. *Progress in Neuropsychopharmacology and biological psychiatry*, 13, 373 – 379

Ovsepian S. V., Anwyl R. and Rowan M.J. (2004). Endogenous acetylcholine lowers the threshold for long-term potentiation induction in the CA1 area through muscarinic receptor activation: in vivo study. *European Journal of Neuroscience* 20(5): 1267-1275.

Hasselmann H. (2014). Scopolamine and depression: a role for muscarinic antagonism? CNS Neurol Disord *Drug Targets*;13(4):673-683. doi: 10. 2174/1871527313666140618105710.

Deaton JG, Nappe TM. (2021). Warfarin Toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 28613764

World Health Organization (2003). Traditional Medicine. Geneva, Switzerland: *World Health Organization*.

Feldman J. M. and M'Lee E. (1985). Serotonin content of foods: effect on urinary excretion of 5-hydroxylindole acetic acid. *The American Journal of Clinical Nutrition* 42: 639-643.

Mosienko, V., Bert, B., Beis, D., Mather, S., Fink, H., Bader, M. and Alenina [2012]. Exaggerated aggression and anxiety in mice deficient in serotonin. *Translational Psychiatry*, 2[5], 122-124.

Paylor, R., Baskall- Baldini, L., Yuva, L. and Wehner, J. M. [1996]. Developmental differences in place-learning performance between C57BL/6 and DMA/2 mice parallel the ontogeny of hippocampal protein kinase C. *Behavioural Neuroscience*, 110, 1415-1425.

Livonen, H., Nurminen, L., Harri, M., Tanila, H. and PouliVali, J. [2003]. Hypothermia in mice tested in Morris Water Maze. *Behavioural Brain Research*, 141, 207-213.

Ennaceur A. and Delacour J. (1988). A new one trial test for neurobiological studies of memory in rats.*Neuroreport8*: 1173-1178.

Brown, R. E., Corey, S. C. and Moore, A. K. [1999]. Differences in measures of exploration and fear in MHC-Congenic C57BU6J and B6-H-2K mice. *Behaviour Genetics*, 26, 263-271.

Podhorna J. and Brown R.E (2002). Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. Genes, Brain and Behaviour 1:96-110.

Sik A., VanNieuwehuyzen P., Prickaerts J. and Blokland A. (2003). Performance of different mouse strains in an object recognition task. *Behavioural Brain Research* 147: 49-54.

Stanley, J. L., Lincoln, R. J., Brown, T. A., McDonald, L. M., Dawson, G. R., & Reynolds, D. S. (2005). The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. *Journal of Psychopharmacology*, 19(3), 221-227.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods*, 11(1), 47-60.

Walther, D. J., Peter, J. U., Bashammakh, S., Hörtnagl, H., Voits, M., Fink, H., & Bader, M. (2003). Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science, 299(5603), 76-76.

Yehuda, S., Rabinovtz, S., Carasso, R. L., & Mostofsky, D. I. (1996). Essential fatty acids preparation (SR-3) improves Alzheimer's patient's quality of life. *International Journal of Neuroscience*, 87(3-4), 141-149.

Bolivar V.J. (2010). Intra-session and inter-session habituation in mice: From inbred strain variability to linkage analysis. *Neurobiology of Learning and Memory* 92(2): 200-214.

Blackford J.U., Allen A.H., Cowan R. L. and Avery S. N. (2013). Amygdala and hippocampus fail to habituate to faces in individuals with an inhibited temperament. *Social Cognitive and Affective Neuroscience* 8(2): 143-150.

Samira. A., Zahra .R., Mahmoud, R (2015). Effects of Matricarria Chamomilla extract on motor coordination impairment induced by scopolamine in rats. *Asian Pacific Journal of Tropical Biomedane* 5(10): 829 – 833.