SRYAHWA PUBLICATIONS

Research Journal of Food and Nutrition
ISSN: 2637-5583 | Volume 8, Issue 1, 2025
https://doi.org/10.22259/2637-5583.0801004

RESEARCH ARTICLE

Effects of Consumption of Cooked Beans (*Phaseolus vulgaris*) as well as Serotonin Precursor Diets on Scopolamine-impaired Memory in Mice

Victory C. Okore[1], Martins J. Uket[1], Chisom C. Ekwonu[2], Onyinye E. Nwachuya[2], Anthony C. Egbo[2], Ifebuche N. Ugwu[2], Odunayo-Bamisi J. Omoghe[2], Chinecherem S. Onah[3], Ndukwe C. Kalu[4], Precious C. Ugwuoke[5], Muhammad S. Mohammed[6], Mohammed Hafeezah[7], Chidimma S. Ngwu[8], John C. Nnamani[2], Sunday J. Imbigbu[9], Calistus D. Kyon[10], Innocent Ugochukwu[2], Ikenna K. Uchendu[2*]

[1]*Department of Human Physiology University of Calabar, Calabar-Nigeria.*
[2]*Department of Medical Laboratory Sciences, University of Nigeria, Enugu Campus, Enugu State, Nigeria.*
[3]*Department of Radiography and Radiological Sciences, University of Nigeria, Enugu Campus, Enugu State, Nigeria.*
[4]*F. Erismann Institute of Public Health, I.M Sechenov First Moscow State Medical University, Moscow, Russia.*
[5]*Department of Food science and technology, Federal polytechnic Oko, Anambra state, Nigeria.*
[6]*Department of Agricultural economics, Ahmadu Bello University Zaria, kaduna state, Nigeria.*
[7]*Science student, Aisha Academy, Zaria, Kaduna State, Nigeria.*
[8]*Department of Medical Laboratory Science Madonna University, Elele Campus, Rivers State, Nigeria.*
[9]*Science Department, Plateau State Polytechnic Academy, Barkin Ladi Plateau State, Nigeria.*
[10]*Department of Medical Laboratory Science, University of Jos, Plateau State State, Nigeria.*

Received: 01 July 2025 Accepted: 04 August 2025 Published: 12 August 2025
Corresponding Author: Ikenna Kingsley Uchendu, Department of Medical Laboratory Science, University of Nigeria Enugu Campus, Enugu State, Nigeria.

Abstract

Background and Objective: Alzheimer's disease (memory impairment) is on the increase worldwide. It has never been established if eating beans plus tryptophan, a serotonin precursor, may help people with memory loss. Thus, the purpose of this study was to examine how scopolamine-impaired learning and memory in mice were affected by the consumption of cooked beans (*Phaseolus vulgaris*) and diets high in serotonin precursors.

Methods and Study Design: Using random assignment, 60 mice were divided into 6 groups (10 mice each); Control, Scopolamine only, Scopolamine with 50% cooked bean diet, Scopolamine with serotonin precursor diet, 50% cooked bean diet only, and serotonin precursor diet only. Prior to studying the learning and memory pattern, initial research was conducted on food and water intake as well as changes in body weight. Learning and memory patterns were investigated using novel object recognition tests and Morris water mazes.

Results: The results showed that preliminary studies on food and water consumption, together with variations in body weight, were significantly lower ($p<0.05$) in the group that received just scopolamine treatment as opposed to the control and other experimental groups. In contrast to the control and other test groups, the scopolamine-only group showed slowed learning ($p<0.05$). In addition, the scopolamine-only group had worse memory than any other experimental group ($p<0.05$).

Conclusion: Mice's learning and memory were enhanced by bean and serotonin precursor diets, while mice's memory was hampered by scopolamine. The serotonin produced from tryptophan in beans may be responsible for the observed improvement in memory.

Keywords: *Phaseolus Vulgaris*.Scopolamine, Alzheimer's Disease, Tryptophan, Beans.

Citation: Victory C. Okore, Martins J. Uket, Chisom C. Ekwonu, *et al*. Effects of Consumption of Cooked Beans *(Phaseolus vulgaris)* as well as Serotonin Precursor Diets on Scopolamine-impaired Memory in Mice. Research Journal of Food and Nutrition. 2025;8(1):47-62.

1. Introduction

A group of symptoms brought on by brain abnormalities is referred to as dementia. According to a 2008 World Health Organisation report, dementia is one of the main causes of disability in older adults (those 65 and older). Global and permanent cognitive deterioration that is severe enough to impair day-to-day functioning is a hallmark of this complicated illness[1]. 60% to 90% of dementia patients have Alzheimer's disease, which is the most prevalent cause of dementia. Vascular dementia, mixed dementia, and dementia with Lewy bodies are the next most common causes of dementia[2, 3].

The preferred medication for impairing memory in mice and other animals is scopolamine. The memory loss or cognitive dysfunction seen after using this medication is comparable to what is seen in persons with dementia. A muscarinic receptor antagonist is scopolamine[4]. Long-term potentiation, which is in charge of long-term memory, is hampered[4, 5]. In order to assess the anti-amnesiac effects of novel medications, it can also be used as an amnesiac.

The negative consequences of synthetic medications have led to a search for safer and more effective natural therapies. Today, 80% of the world's population uses traditional medicine for some basic health care, including mental health, according to a World Health Organisation data analysis[6–9]. In light of this, natural items could offer a fresh supply of advantageous neuropsychotropic medications if their processes are well established and they receive scientific validation.

Numerous natural products or plants have been discovered to constitute effective resources for both traditional and modern medicine. Herbal medicine has been shown to have genuine utility[10]. The majority of indigenous people in developing nations use local plants to treat a variety of illnesses because modern western medicine is costly. Various medicinal plants are used in Nigerian herbal recipes to treat a variety of illnesses[9].

The dicotyledonous common bean (Phaseolus vulgaris) is a member of the pea family (Gatel, 1994). In many regions of the world, common beans form a staple diet[11]. Protein, carbs, dietary fibre, minerals, vitamins, and a variety of phenolic chemicals are all abundant in beans[12]. The significant antioxidant activity found in beans is currently of special interest to researchers. It is not surprising that nutritionists would describe beans as a virtually perfect food because they are a very nutritious food in many ways[11, 12]. Other chemical substances found in beans include flavonoids, glycosides, tannins, and saponins. Serotonin is one of the several phytochemical elements that has neurobehavioral effects on mood, memory, learning, and sleep[13]. It has been demonstrated that serotonin functions as a neurotransmitter to modify behaviour in response to shifting stimuli, influencing learning, pharyngeal function, pumping locomotion, and egg laying via acting on neurones and muscles [14].

Serotonin has been shown to improve memory and learning. Long-term bean eating enhances learning and memory in mice that appear to be normal, according to Osim *et al*. [15]. It has not been determined whether giving mice with memory impairment a bean diet high in serotonin and its precursor 5-hydroxytrytophan (5-HTP) also enhances their learning and memory. In light of this, the aim of this study is to ascertain whether giving mice cooked beans and diets high in serotonin precursors can help them recover from scopolamine-induced memory impairment.

2. Materials and Methods

2.1 Materials

For the purpose of this research, the following materials were utilized: Common bean (*Phaseolus vulgaris*), Serotonin precursor diet (5HTP), Scopolamine (memory impairment drug), Weighing balance, Electric blender, Animal home cages, Masking tape, Syringes and needles, Water, Feeding trough, Stopwatches, Permanent marker, Novel Object Recognition Task (NORT) maze, Morris water maze, Rodent chow, Tempura paint, Tissue paper, Methylated spirit, Disinfectant

2.2 Method

2.2.1 Experimental Animals

The study employed sixty (60) adult CD1 white mice, weighing between 17g and 26g that were acquired from the Physiology Department's animal home at the University of Calabar in Calabar. They were divided into six (6) groups of ten (10) mice each by random assignment. Each study group's mice were kept separately in a plastic cage with a wire screen on top and an iron gauze bottom grid. In addition to having a 12-hour natural light-dark cycle, the animal chamber was properly aired and maintained at ambient temperature and humidity of 22 ± 3^0C and 40-70%, respectively.

2.2.1.1 Ethical Approval

As outlined in the Guidelines for the Care and Use

of Laboratory Animals, all animals were treated in accordance with the rules for animal research. The University of Calabar's Faculty of Basic Medical Science research ethics committee granted ethical approval for this study, with the Ethical Right Permission Number: FAREC/PA/010BC31012.

2.2.2 Experimental Design

A digital weight balance was used to weigh the mice. Since each animal was housed separately, identification cards were simply used to identify the animals. They were divided into six (6) groups of ten (10) mice each by random assignment:

Group 1 is the control

Group 2 is scopolamine only

Group 3 is scopolamine and cooked bean diet

Group 4: scopolamine and serotonin precursor diet

Group 5: diet consisting solely of cooked beans

Group 6: diet consisting solely of serotonin precursors

The experiments lasted thirty-eight (38) days and involved a total of sixty (60) mice. The mice weighed between 17g and 26g and were between 30 and 35 days old. After undergoing clinical and neurological testing, it was determined that none of the animals had any systemic diseases.

2.2.3 Treatment Regimen

All animals had unrestricted access to food and water. Standard normal rodent chow obtained from Goldie market, Calabar, Cross River State's local market, was used to feed the animals. By regularly cleaning and clearing the cages of excrement and spilt feed, good hygiene was maintained.

2.2.3.1 Preparation of Beans

A total of twenty (20) cups of beans were purchased from Marian Market, a Calabar local market, Cross River State. An electric blender was used to grind the beans into a powder after they had been cooked and allowed to air dry. It weighs 1,560g in powdered form.

2.2.3.2 Preparation of Beans Diet

A 50:50 (w/w) ratio of beans to normal rodent chow was achieved by combining 1000g of powdered cooked beans with 1000g of rodent chow. A bending machine was used to mix the constituent into a homogenous mixture.

2.2.3.3 Preparation of Serotonin Precursor Diet

For this research, a serotonin precursor (5-hydroxy tryptophan) was purchased from Sigma Aldrich. Using the Feldman and M'Lee (1985) method, which Mosienko *et al*.[16] modified, the amount of powdered 5-hydroxytrptophan (a precursor to serotonin) in cooked beans was estimated. To create the serotonin precursor diet, 115 mg (1.15 g) of the precursor was combined with 98.85 g of feed. In order for the 5 HTP that was added to be equal to the amount of cooked beans (100 g). To create the serotonin precursor diet, the mixture was blended using an electric blender.

2.2.4 Administration of Diets and Drugs

The mice in Group (I) were given regular rodent chow and 0.1mg/kg of normal saline. (IP).

The mice in Group (II) were given regular rodent chow and 0.1mg/kg of the scopolamine. (IP).

The mice in Group (III) were fed with cooked beans diet as well as 0.1mg/kg of scopolamine. (IP).

The mice in Group (IV) were fed with serotonin precursor diet as well as 0.1mg/kg of scopolamine. (IP).

The mice in Group (V) were feed with cooked beans diet as well as 0.1mg/kg of normal saline. (IP).

Group (VI) mice were feed with serotonin precursor diet as well as 0.1mg/kg of normal saline. (IP).

(IP: Intraperitoneally)

2.2.5 Determination of food, water intake and Body Weight

By measuring the amount of food that remained in the container after a period of 24 hours and deducting it from the original amount at the beginning of the day's feeding, the daily food intake was calculated. The amount of water left over after 24 hours of feeding was deducted from the original amount in the drinking containers to determine the daily water intake. That day's consumption was the difference. Every day, the animal's weight was recorded in order to monitor any changes. All animals were given unlimited access to food and water. Daily food and drink intake was tracked, and weekly body weight changes were measured.

2.3 Apparatus and experimental protocol

2.3.1 Morris Water Maze (MWN)

In this research, learning and memory were studied using a modified Morris Water Maze (MWM) for

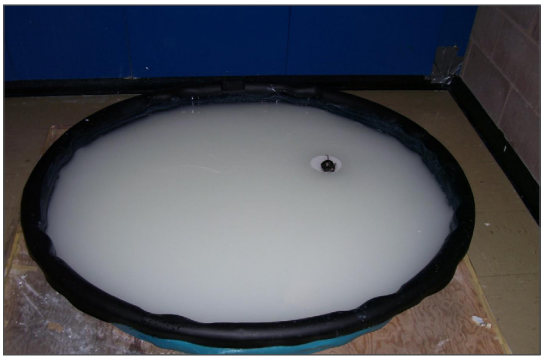

PLATE 1. *Morris Water Maze (MWM)*

mice[17], which is smaller than the maze designed for rats. The circular polypropylene pool, also known as the "Pelican" pool from Canadian tires, which is 110 cm in diameter and 20 cm deep, is used to create the water maze. The pool is filled with room-temperature tap water up to a depth of 14 cm (0.5 cm over the platform), and 100 mL of non-toxic white liquid tempura paint (Schola, Marieville) is added to make it opaque. The water is allowed to sit overnight until it reaches room temperature, which is 22 ± 1°C.

The northwest, northeast, southwest, and southeast quadrants make up the pool. Masking tape is used to indicate the boundaries of these quadrants, which are North, South, East, and West, on the pool's sides. A 13.75 cm by 9 cm Plexiglas cylinder serves as the maze's escape platform. To make it heavy in the water, cement has been placed inside the cylinder. A colourful flag is set up in the middle of the platform, which has a detachable red and yellow striped top that measures 3 cm by 9 cm in diameter. A visible escape platform is created for visible platform tests by adjusting the pool's water level to 0.5 cm below the striped top's surface, while a concealed escape platform is created by adjusting the water level to 0.5 cm above the white cylinder (with the striped top removed).

The room where the pool is situated measures 5.2 x 2.4 meters. The room's walls are covered in a number of posters that serve as visual cues. The room's furniture, including the table, chairs, and washbasin, also serves as visual signals. The room is faintly lighted with diffuse white light (30 lux) while testing is taking place. The water maze's animal performers are meticulously documented.

There are many different procedures for testing mice in the Morris Water Maze (MWM). It takes eight (8) days to test in the water maze according to our paradigm:

Day 1: first day of acquisition

Day 2: second day of acquisition

Day 3: third day of acquisition

Day 4: first day of reversal

Day 5: second day of reversal

Day 6: third day of reversal

Day 7: trial of the probe

Day 8: the day for visible-platform

The hidden platform is used for both acquisition and reversal training (the water is 0.5 cm above the platform). The platform is transferred to the other side of the maze during reversal. To evaluate visuospatial memory, there is no escape platform during the probing trial. The platform is shifted to a different pool quadrant and the visible top is added on the visible-platform day. This evaluates motivation to find the platform as well as basic visual ability.

Every day, the mouse is taken out of its home cage and put in a sanitized holding cage devoid of woodchip bedding. To help the mice dry more rapidly, paper towels are ripped into strips and put in the bottom of the holding cages. When this paper towel gets wet, it is changed. The mice are run in groups of four to six, with an inter-trial period of five minutes between each trial. Shorter inter-trial intervals (ITIs) should be avoided because they can cause mice to perform worse because they are hypothermic.

The platform is positioned in the Northeast quadrant's centre during acquisition training. Four trials are given to each mouse each day. The mouse has up to 60 seconds in each trial to find the escape platform. A Latin square design is used to predetermine the mice's starting places, preventing the duplication of starting location sequences on consecutive test days. The borders of the quadrants (West, North, East, or South) were potential starting points. To reduce handling stress, a small, sterile 500-mL plastic container is used to take each mouse from its holding cage for each trial. At the appropriate starting position, the animal is then submerged in the water.

After that, the mouse is given 60 seconds to explore

the pool as it searches for the hidden escape platform. The mouse is permitted to remain on the platform once the animal finds it, and the countdown is manually paused. The mice are allowed to observe the extra-maze environment for ten seconds once they are on the platform. The plastic container is used to direct the mouse onto the platform if it is unable to locate it within the given time. The process is then repeated with the subsequent mouse in the pool. Over the course of three days, each animal completes four trials every day for a total of twelve acquisition training sessions, each starting from a different one of the four locations.

On the fourth day, reversal training starts. Mice are once more assigned to their proper starting positions once the invisible platform is moved to the opposite quadrant (the southwest quadrant). The steps involved in reversal training are identical to those in acquisition training. The animals complete a total of 12 reverse training trials, with each animal completing 4 trials daily for 3 days.

On the seventh day, a probing trial is used to evaluate visuospatial memory. The maze does not currently have an escape platform. The time spent in each quadrant of the maze is recorded after each mouse is given 60 seconds to explore the pool from one of the four potential start places. After 60 seconds, the mouse is removed using the container and allowed to dry in a holding cage before being put back in its own cage.

On the eighth day, the visible platform task is carried out. The platform that is visible has been moved to the pool's Northwest quarter. Four trials are completed by the mice using the identical protocols as in acquisition and reversal training.

The following actions were assessed during visual, reversal, and acquisition training:

1. Swim distance
2. Average velocity
3. Swim latency (time to locate and ascend the escape platform)
4. The frequency and duration of thigmotaxic behavior, which involves swimming in a 9-cm-wide corridor around the pool's edge
5. Swim path error, which is calculated by measuring how long it takes the mouse to travel a relatively direct path from the start position to the hidden platform's location (Whishaw, 1985).

The subject is positioned in the pool five times per second, allowing for the recording of its distance from the escape platform in averages of one second. This approach reflects search error (differences from an ideal search) in the closeness scores that are computed.

The measurements taken during the probing trial are:

1. The number of entries into the Northeast, Northwest, Southeast, and Southwest quadrants
2. How much time was spent in each quadrant
3. The frequency with which the mouse crosses the platform's location during reversal training (also known as annulus reversal crossing)
4. The quantity of times, during acquisition training, the mouse passes the platform's location (annulus acquisition crossing)
5. How long and how often thigmotaxic conduct occurs (9 cm corridor width)
6. Proximity to the platform location.

2.3.2 The Novel Object Recognition Task (Open Field)

Rats are known to spend more time exploring new objects than familiar ones, which led to the creation of the novel object recognition task (NORT) as a declarative memory test.

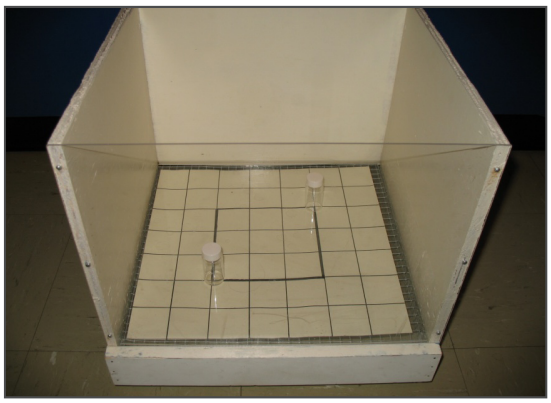

PLATE 2. *Open field apparatus for NORT*

Since then, its validity as a test of mice's recognition memory has been confirmed[18]. This test which has many variants is based on a spontaneous behavior in animals – an unconditioned preference for novel objects. This study employed the modified approach of Brown *et al*.[19]. Compared to other learning and memory tests, the NORT is less stressful for the mouse because it doesn't require food deprivation like the radial arm maze or swim stress like the Morris water maze. This is advantageous because stress can impair memory function.

2.3.2.1 Procedure

Before the test started, all of the mice were given five minutes to get acquainted with the equipment. Mice in their home cages were transported to the test room. Using a little container, they were transferred from their home cages to the testing equipment and back again. Between trials, the mice were put back in their cages after each 5-minute trial, and the equipment was cleaned with 70% ethyl alcohol and allowed to dry.

Depending on how long it takes for the items to be retained between training (trial 1) and testing (trial 2), there are two methods for doing the new object test, each of which targets a distinct kind of memory. Two identical object pairs are required. There is a little interval between the novel objects' introduction and testing in the first approach. The acquisition and recognition trials were place on the same day, with a retention period of five, fifteen, thirty, or one hour between each trial. In the initial test, two identical items (O1 and O2) were positioned in the open field across from one another in diagonal corners. Items were attached to the apparatus's floor using reusable glue. After being removed from its home cage, the mouse was set up in the centre of the open field arena. For five minutes, each mouse was free to explore the arena and its contents. The mouse is taken out of the device and put back in its cage at the conclusion of the experiment. The mouse was put back into the test equipment (trial 2) after a 15-, 30-, or 1-hour inter-trial interval (retention time). The arena now has a new item (N) in place of O1 or O2, and the familiar object (O1 or O2 from trial 1) in one of the two locations in trial 1. Trial 2 involved five minutes of recording the same behaviors as trial 1.

2.3.2.2 Behaviors scored

During the Open Field (NORT), the following behaviors were scored:

1. Line Crossing: how often the mice used all four paws to cross one of the grid lines.
2. Rearing: how often the mice in the maze stood on their hind legs.
3. Against a Wall: how often the mice stood on their hind legs in front of an open field wall.
4. Stretch Attend Postures: how frequently the animal extended its head and shoulders forward before retracting to its starting position.
5. Grooming: how often and how long the animal licks or scratches itself when it is motionless.
6. Approaches to Everything: contacting the thing with the nose or aiming it at it from a distance of less than 1 cm.
7. The amount of time spent climbing or sniffing each thing. It is not regarded as an exploratory behavior to sit on the object.

2.4 Statistical Analysis

The data gathered was presented as mean ± SEM. Analysis of variance (ANOVA) and a post HOC test (least square difference or "LSD" test) were used to assess experimental data in order to identify significant differences between means. The SPSS 18 statistical software was used for the analysis. At $p<0.05$, the mean values were determined to be significant.

3 Results

3.1 Mean Daily Food Intake

The mean daily food intake for the experimental groups of control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet was 3.09 ± 0.02, 3.08 ± 0.37, 3.31 ± 0.03, 3.30 ± 0.03, 3.30 ± 0.03, and 3.30 ± 0.20, respectively, as shown in Figure 1. The scopolamine test group's mean food intake was significantly lower ($p<0.05$) than that of the control and other experimental test groups. The control group did not differ significantly from the other experimental groups.

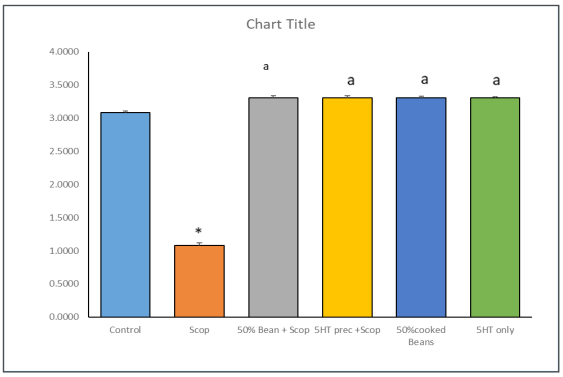

Figure 1. *Comparison of food intake of control, 50% beans, 5HT prec. and scopolamine treated groups. The values are given as follows: a = p<0.05 vs scopolamine; * = p<0.05 vs control; mean + SEM, n = 10.*

3.2 Mean Water Intake

3.48 ± 0.08, 2.34 ± 0.06, 3.55 ± 0.04, 3.43 ± 0.14, 4.55 ± 0.12, and 4.44 ± 0.11 are the average water intakes for the experimental groups, respectively, for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet. Comparing the scopolamine test group to the control and other experimental test groups, the mean water intake was significantly lower ($p<0.05$). When comparing the other experimental groups to the control, no discernible differences were seen. The outcome is depicted in figure 2 below.

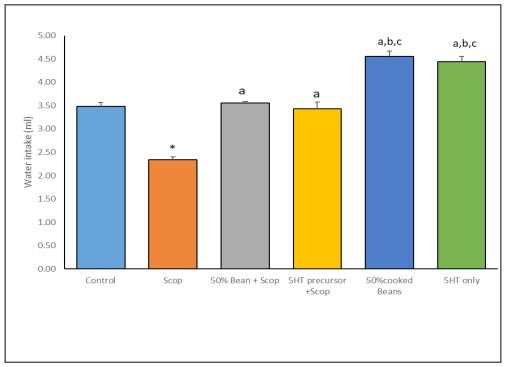

Figure 2. *Comparison of water intake of control, 50% cooked beans, 5HT precursor andscopolamine-treated groups. The following values are reported: a = p<0.05 vs scopolamine, b = p<0.05 vs 50% cooked beans + scopolamine, and c = p<0.05 vs 5HT precursor + scopolamine. The mean + SEM, n = 10.* = p<0.05 vs control*

3.3 Mean body weight change

The mean changes in body weight for the experimental groups controlling, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet are 2.66 ± 0.239, 1.23 ± 0.119, 2.83 ± 0.323, 2.88 ± 0.244, 3.03 ± 0.388, and 3.02 ± 0.378, respectively, as shown in Figure 3 below. The scopolamine test group's body weight was significantly lower ($p<0.05$) than that of the control and other experimental test groups. The control group did not differ significantly from the other experimental groups.

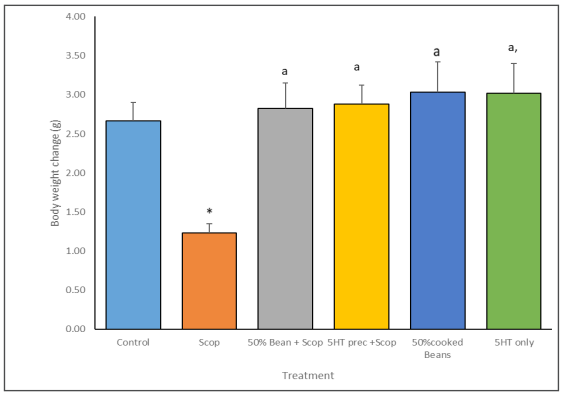

Figure 3. *Comparison of body weight change of control, 50% beans, 5HT prec. and scopolamine treated groups. The values are given as follows: a = p<0.05 vs scopolamine; * = p<0.05 vs control; mean + SEM, n = 10.*

3.4 Behaviours Scored In Morris Water Maze

3.4.1 Comparison of Swim Latency during the Acquisition Training

The swim latencies between the control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet during the acquisition training of the Morris water maze (Days 1, 2, and 3) are displayed in Figure 4. In the first day, the swim latencies for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet 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, respectively. The findings indicate that, on day 1, there was no discernible difference between the experimental groups and the control group. On the other hand, 50% cooked beans' swim latency on day 2 (38.26 ± 3.534 sec) was significantly lower than that of the control and other experimental groups ($p<0.05$). When compared to the control and other experimental groups, the swim latency of the scopolamine-only group on acquisition day 3 was noticeably greater (47.41 ± 3.088 sec).

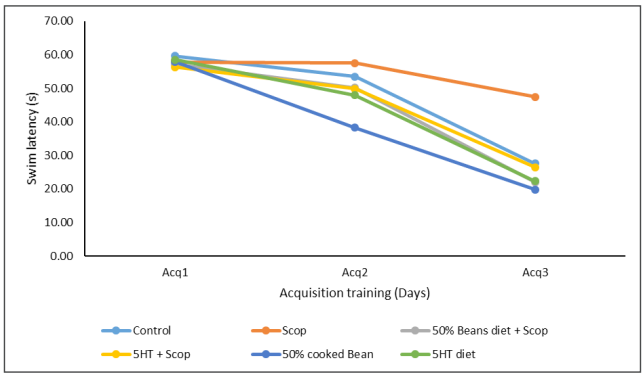

Figure 4. *Comparison of acquisition training of control, 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris Water Maze (MWM). Value are shown as mean +SEM, n = 10.*

3.4.2 Comparison of Swim Latency during the Reversal Training

The swim latency curves for the control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet during the reverse training of the Morris water maze (Days 4, 5, and 6) are displayed in Figure 5 below. The results indicate that the 50% cooked beans diet group's swim latency (39.70 ± 3.822) on the first day of reversal training was considerably ($p<0.05$) shorter than that of the control and other experimental groups. On day 2 of reversal training, a similar pattern was noted. During the third day of reversal training, the swim latencies for control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet 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, respectively. Compared to the control and other experimental groups, the scopolamine-only group was noticeably longer ($p<0.05$). Additionally, the results indicate that the experimental groups who were fed cooked beans and a serotonin precursor diet had a significantly shorter delay than the control group ($p<0.05$).

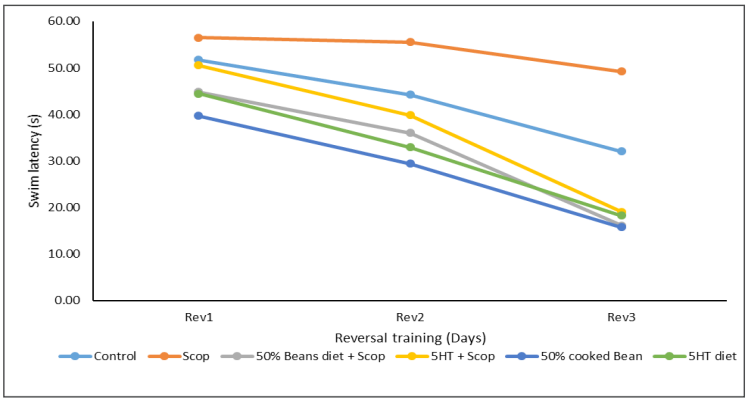

Figure 5. *Comparison of reversal training of control, 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris Water Maze (MWM). Value are shown as mean +SEM, n = 10.*

3.4.3 Comparison of Quadrant Duration

Figure 6 below demonstrates the comparison of the quadrant time on the Morris Water Maze (MWM) test's probing trial day. The mean durations 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 shows that, the groups treated with control, cooked beans and serotonin precursor diets has greater preference in the northeast and southwest quadrants compared to the group treated with scopolamine only ($p<0.05$).

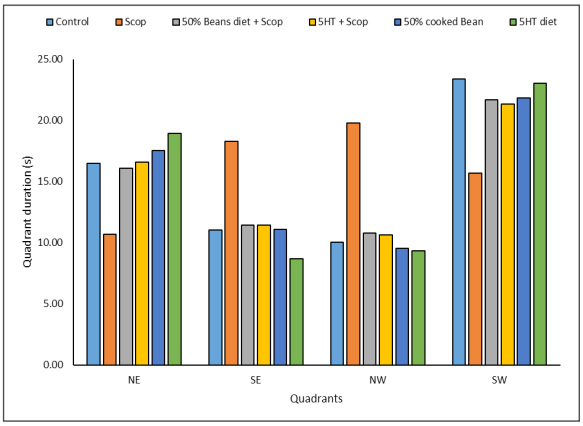

Figure 6. *Comparison of quadrant duration during probe trial test of control, 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris Water Maze (MWM). Value are shown as mean +SEM, n = 10.*

3.4.4 Duration of Quadrant Retention

In the probe trial of the Morris water maze test, Figure 7 below compares the quadrant duration retention between the Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet. The corresponding durations were 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. The findings indicate that the mice administered with scopolamine alone had considerably worse short-term retentive memory than the control and other experimental groups ($p<0.05$). The other experimental groups did not differ significantly from the control group.

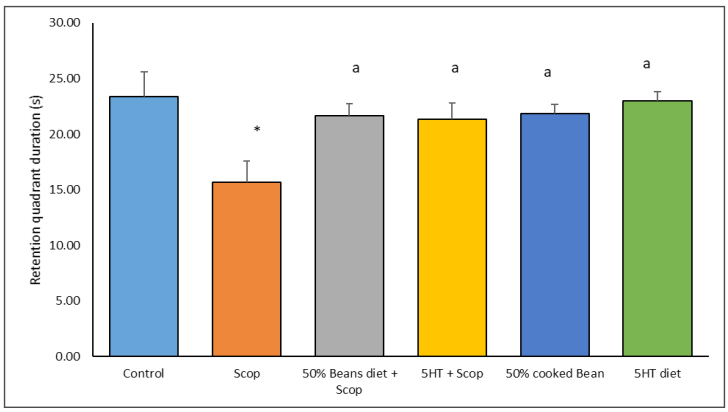

Figure 7. *Comparison of the control time in the Retention quadrant 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris Water Maze (MWM). Mean + SEM, n = 10, * = $p<0.05$ vs. control, a = $p<0.05$ vs. scopolamine are the values displayed.*

3.4.5 Annulus Acquisition Frequency

During the probe trial task frequencies, the annulus acquisition crossing 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, respectively, Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet. According to the results, the scopolamine-only group's annulus acquisition crossing was significantly reduced when compared to of the control and other experimental groups ($p<0.05$). The experimental groups did not differ significantly from the control group. The outcome is shown in Figure 8 below.

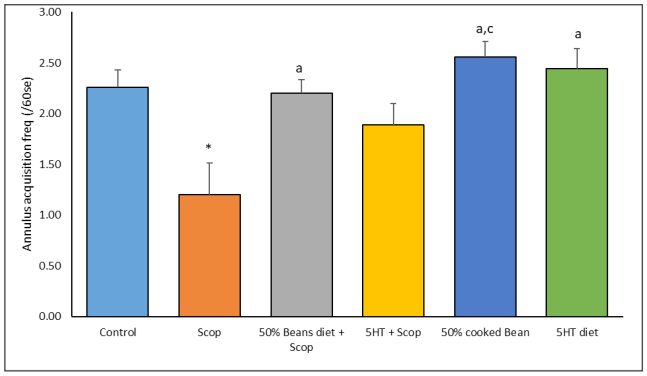

Figure 8. *Comparison of annulus acquisition training of control, 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris Water Maze. Values are shown as follows: a = p<0.05 vs scopolamine, c = p<0.05 vs 5HT precursor + scopolamine, * = p<0.05 vs control, and mean + SEM, n = 10*

3.4.6 Annulus Reversal Crossing Frequency

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 were the respective annulus reversal crossing frequencies for the control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet during the probe trial task. The annulus reversal crossing was significantly reduced in the scopolamine-only group than in the control and other experimental groups, according to the results ($p<0.05$). Reversal crossing is also significantly higher in the 50% cooked beans only group than in the control and other experimental groups ($p<0.05$). The outcome is shown in Figure 9 below.

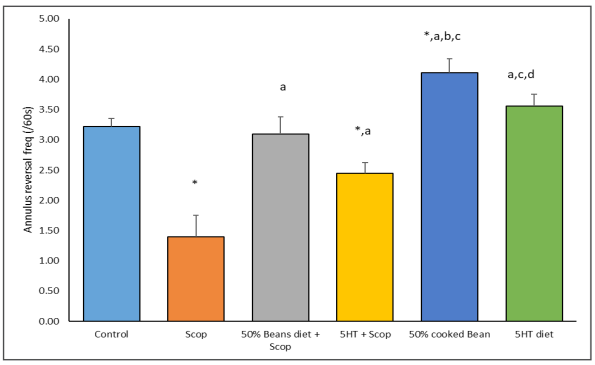

Figure 9. *Comparison of annulus reversal frequency of control, 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris water maze. * = p<0.05 vs control, a = p<0.05 against scopolamine, b = p<0.05 vs 50% cooked beans + scopolamine, c = p<0.05 vs 5HT precursor + scopolamine, and d = p<0.05 vs 50% cooked beans. The values are shown as mean + SEM, n = 10.*

3.4.7 Comparison of swim latency on the visible platform test

The swim latencies during the visible platform test for Control, scopolamine, 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 latencies of the result the swim latency for the scopolamine only group was significantly longer compared to control and the other experimental groups ($p<0.05$). The 50% cooked beans only group, from the result shows significant shortest latency during the visible test in comparison to the control and the other experimental groups ($p<0.05$). See Figure 10 below.

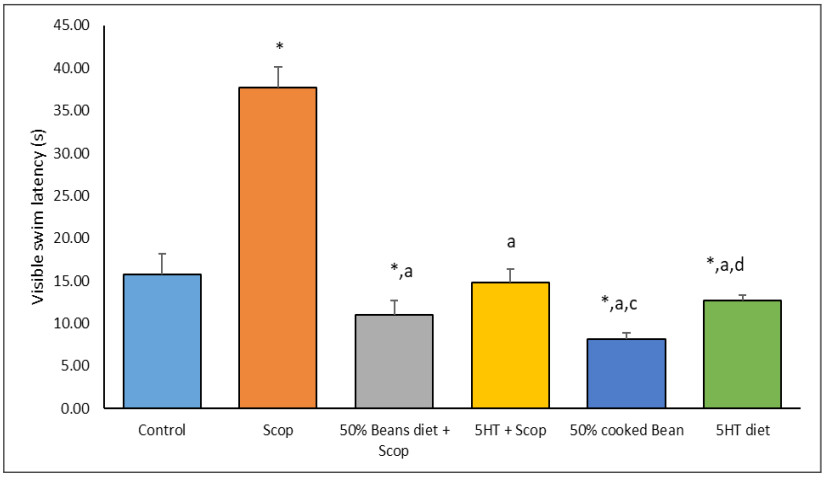

Figure 10. *Comparison of visible swim latency of control, 50% cooked beans, 5HT precursor and scopolamine treated groups in Morris water maze. Results are shown as mean + SEM, n = 10, * = p<0.05 vs control; a = p<0.05 against scopolamine, b = p<0.05 vs 50% cooked beans + scopolamine, c = p<0.05 vs 5HT precursor + scopolamine, and d = p<0.05 versus 50% cooked beans.*

3.5 Behaviours scored in novel object recognition test

3.5.1 Comparison of the Habituation Index for Short Term Memory

Figure 11 shows the mean habituation index for short term memory of the Control, scopolamine, 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. Comparing the Scopolamine-only group to the control and other experimental groups, the habituation index for short-term memory was significantly lower (p<0.05). The other experimental groups and the control group did not differ significantly.

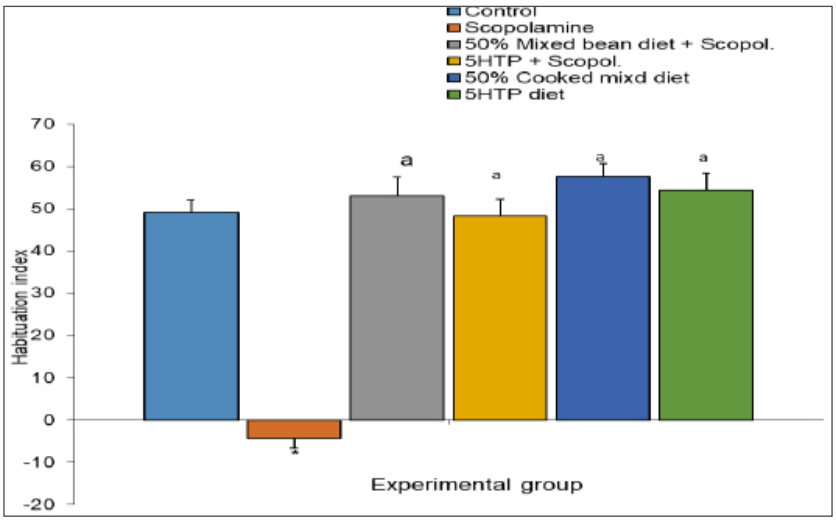

Figure 11. *Comparison of The Habituation Index for short Term Memory during the NORT in control, 50% Cooked Beans, 5HT precursor and scopolamine treated groups. Values are expresed as mean + SEM, n = 10. = p<0.05 vs control, a = p<0.05 vs scopolamine*

3.5.2 Comparison of The Habituation Index for Long Term Memory

Figure 12 below shows the mean habituation index for long term memory of the control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet are 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. In comparison to the control and other experimental groups, the Scopolamine-only group's habituation index for long-term memory was significantly lower (p<0.05). When compared to the control group, the mean values of the groups fed cooked beans exclusively and the serotonin precursor diet solely were significantly higher (p<0.05).

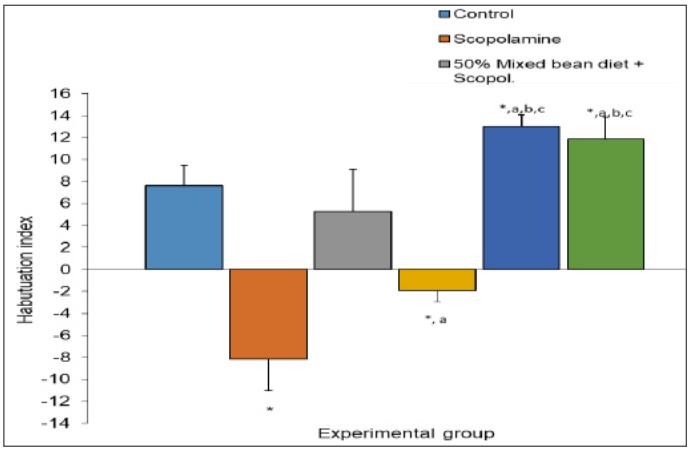

Figure 12. *Comparison of The Habituation Index for long Term Memory during the NORT in control, 50% Cooked Beans, 5HT precursor and scopolamine treated groups. Values are expresed as mean + SEM, n = 10. = p<0.05 vs control, a = p<0.05 vs scopolamine, b= 50% cooked mixed diet, c=p<0.05 vs mixed bean diet + Scopol*

3.5.3 Comparison of The Discrimination Index for Short Term Memory

The mean discrimination index for short term memory of the Control, scopolamine, 50% cooked beans + scopolamine, 5HT precursor diet + scopolamine, 50% cooked beans, and 5HT precursor diet are 0.43 ± 0.04, -0.18 ± 0.05, 0.69 ± 0.11, 0.52 ± 0.06, 0.64 ± 0.03 and 0.52 ± 0.05 respectively. The discriminative index value for scopolamine only group was significantly lower compared to control and other experimental groups (p<0.05). The result also shows that, there was no significant difference between the other experimental groups when compared to control. See Figure 13 below.

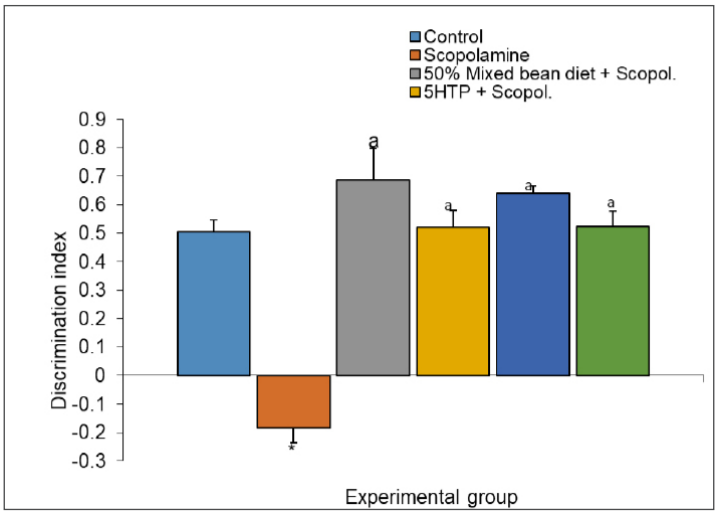

Figure 13. *Comparison of Discrimnative Index for Short Term Memory during the NORT in control, 50% Cooked Beans, 5HT precursor and scopolamine treated groups. Values are expresed as mean + SEM, n = 10. = p<0.05 vs control, a = p<0.05 vs scopolamine*

3.5.4 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 are 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 (Figure.14).

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 has longer discrimination index compared to control and other experimental groups (p<0.05). The groups treated with 50% cooked beans diet + scopolamine and serotonin precursor diets + scopolamine were significantly decreased compared to control (pp<0.05).

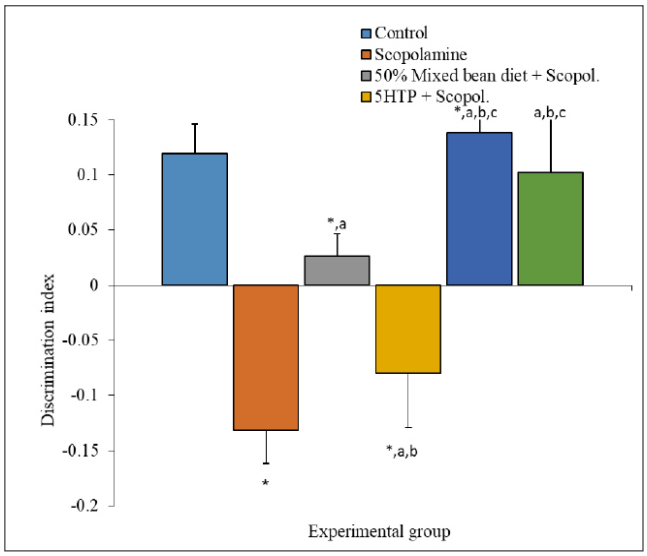

Figure 14. *Comparison of Discrimnative Index for long Term Memory during the NORT in control, 50% Cooked Beans, 5HT precursor and scopolamine treated groups. Values are expresed as mean + SEM, n = 10. = p<0.05 vs control, a = p<0.05 vs scopolamine, b= 50% cooked mixed diet, c= p<0.05 vs 50% of miced bean diet + Scopol*

4. Discussion

In this study, the effects of serotonin precursor meals and cooked beans (Phaseolus vulgaris) on mice with scopolamine-impaired memory were investigated. The neurobehavioural effects of serotonin precursor diet was also compared with cooked beans diet because, it is one of the constituents of beans. Learning and memory were the parameters considered in this study while the Morris Water Maze (MWM) and the Novel Object Recognition Test (NORT) were used in the study.

Compared to the control and other experimental groups, mice treated with scopolamine alone had a considerably decreased mean daily food intake ($p<0.05$). The other test groups did not differ significantly from the control group. The lateral hypothalamic nucleus regulates the intake of food and fluids, making it the hunger centre. As a result, when an animal is stimulated, it consumes large amounts of food and liquids. However, the animal stops feeding when triggered since the ventromedial hypothalamus nucleus is the satiety centre. There is a chance that scopolamine could stimulate the ventromedial hypothalamus nucleus, resulting in satiety and a reduction in food intake. When comparing the groups fed cooked beans and serotonin precursor diets to the control group, there was no increase in food intake. Prior research indicates that serotonin reduces appetite by modulating dopamine function, which supports the findings of this study[20].

The average water consumption follows the same pattern as the average food intake. Osmoreceptors or thirst receptors in the hypothalamus regulate water intake. When there is a high blood concentration of electrolytes (osmolarity), the osmoreceptors increase thirst[21]. Inhibition of this centre, on the other hand, decreases thirst. Therefore, scopolamine, an anticholinergic medication, is probably to blame for the suppression of the thirst centre.

By the end of the experiment, the scopolamine test group's mean body weight change has significantly decreased ($p<0.05$). The groups fed serotonin precursor diets and cooked beans did not differ in weight from the control group. This finding aligns with the previous research conducted by Livesey *et al.*[22], which found that consuming beans aids in restoring normal body weight.

Visuospatial learning and memory are tested in the hidden platform variation of the Morris water maze. Injuries to the hippocampus hinder this mechanism. The visuospatial learning exercise uses extra-maze cues, while the visible (cued) platform uses a special intra-maze visual cue that is positioned at the escape platform's location. In the cued platform or visuospatial learning test, the swim latency was the amount of time it took the mice to find the hidden platform. The learning process is better when the swim delay is less. In a short amount of time, mice with faster learning speeds were able to recognise the hidden platform's spatial placement and position before their counterparts. A steeper gradient of swim latencies over the three days of acquisition or reversal training also results in a superior learning curve and, consequently, learning.

Swim latencies for the first three days of acquisition training after consuming cooked beans and serotonin

precursor diets revealed that all groups fed these diets had significantly lower swim latencies than the control group and the group that received only scopolamine treatment.

During the reversal training days, the similar pattern was noted. This indicates that these mouse groups learnt more quickly than the control and scopolamine-only groups being fed regular rodent chow because they were able to find the hidden platform more quickly. The learning curve was worse for the scopolamine-only group.

The probe trail in the Morris water maze challenge evaluated visuospatial memory. It is anticipated that during reversal training, mice with a strong recollection of the spatial location and position of the hidden platform would spend more time investigating the quadrant containing the platform during the probe trail (60 seconds of exploration without the hidden platform). In this instance, South-West (SW) was the retention quadrant. The mice in the control group and those fed cooked beans and serotonin precursor meals spent a lot more time exploring the retention quadrant than the mice in the scopolamine-treated group. This demonstrated their superior memory over the mice in the scopolamine-treated group.

The cued Morris water maze evaluates the animals' visual integrity and cued learning. Rather than spatial learning, impairments in the hidden platform model may be caused by sensory motor factors, medications, or brain injuries that impact escape drive. This is controlled by this cueing process, where the escape platform rises above the water's surface. Here, comparisons were also made using swim latencies. Better healed learning is shown by shorter swim latencies in the visible platform task. Poorer cued learning is indicated by longer swim latencies.

In comparison to the mice in the control and scopolamine-only treatment groups, the mice who were fed both cooked beans and serotonin precursor diets exhibited considerably shorter swim latencies. This indicates that mice's learning process and visual integrity were enhanced by eating cooked beans and diets high in serotonin precursors.

In addition to serotonin (5-HT_), tryptophan, and the precursor 5-Hydroxytryptophan (5-HTP), beans are a good source of vitamin B6 [23]. Using vitamin B6 as a co-enzyme, the enzyme aromatic acid decarboxylase transforms tryptophan into 5-HTP, which is then transformed into serotonin (5-HT) by tryptophan hydroxylase. A neurotransmitter called serotonin has been shown to enhance cognitive processes, including memory and learning [23, 24]. Additionally, the inclusion of chemical and mineral constituents including glutamic acid, magnesium, potassium, phosphorus, and calcium, which are known to increase memory and learning, further enhances the ability of beans—both cooked and uncooked—to boost learning and memory.

The discovery that rats will investigate a new object more than a known one led to the development of the novel object recognition task (NORT) for rats as a declarative memory test[25]. Since then, it has been confirmed to be an accurate assessment of mice's recognition memory[18, 19]. Because it doesn't involve any outside incentives, rewards, or punishments, but only a small amount of training and habituation, and because it can be finished quickly, the NORT particularly attractive[26]. The novel object identification task has an advantage over other learning and memory tests in that it requires less stress from the mouse (no food deprivation, unlike the radial arm maze, or swim-stress, like the Morris water maze), which can affect memory performance[27].

There is an initial habituation to the apparatus prior to the NORT, and then two trials in the novel object task, the trial 1: which is an acquisition trial (for short term memory) and the trial 2: which is a retention trial (for long term memory). Index of habituation and index of discrimination were measured. Intrasession and intersession habituations represent short and long term habituations respectively.

From the results obtained the habituation index for short term memory for Scopolamine only treated group was lower in comparison to the other experimental groups, whereas the values from the groups fed with cooked beans and serotonin precursor diets groups appeared higher. Decrease in habituation depicts deficit in associative learning[28].

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 mice fed with cooked beans and serotonin precursor diets appeared to be higher than that of the Scopolamine only treated group. This increased habituation could be interpreted as improvement in the memory capabilities of these animals[29].

The short term memory capabilities of group of mice fed with cooked beans diet was the highest as shown by the significant increase in intrasession habituation,

however the animals fed serotonin presursor diet had improved long term memory as shown by the significant increase in intersession habituation index.

It is possible to distinguish between new and familiar objects thanks to the discrimination index. While a higher negative score denotes more time spent with the familiar object, a higher positive score implies more time spent with the novel object[26]. To account for variations in the amount of time spent examining each object, the discriminative index can also be defined as the ratio of the overall exploration time26.

The results showed that, in comparison to control and other experimental groups, the discrimination index for short-term memory of the group that received only Scopolamine was significantly lower, although the values for the other treated test groups did not differ significantly from control. Nonetheless, the long-term memory discrimination index result revealed that the animals fed both cooked beans and serotonin precursor diets had higher values than the group that was only given Scopolamine, with the 50% cooked beans diet group displaying the highest values. The greater positivity of these groups suggests that recognition memory has improved as a result of spending more time investigating the new object. According to these findings, mice's diets of cooked beans and serotonin precursors enhanced their short- and long-term memory, respectively. The antioxidants in the cooked bean diet and serotonin may be responsible for the reported improvement in memory that occurs after consuming the diet.

5. Conclusion

Cooked bean consumption and serotonin precursor diets enhanced memory and learning, but had no effect on the mice's changes in body weight or food and water intake. Tryptophan, which is produced in beans and found in serotonin precursor diets, may be one of the active components underlying these effects that are linked to cooked beans and these diets. In order to enhance mental health in communities around the world and control Alzheimer's disease, the serotonergic potential of cooked beans may be used in the prevention, treatment, and management of mental diseases.

Author Contributions

V. C.O. and M.J.U: Investigation, Resources, Supervision, Funding acquisition, Writing—review & editing. V. C.O.: Methodology, Data curation, Visualization, Formal analysis, Writing—origina, draft. V. C.O.: Writing—review & editing, Sample collection. V.C.O.: Sample collection. V.C.O. and M.J.U: Supervision. V.C.O., M..J.U., C.C.E., O.E.N., A.C.E., I.N.U., O.J.O.,C.S.O., N.C.K.,P.C.U., M.S.M., M.H., C.S.N., J.C.N., S.J.I., C.D.K., I.U., and I.K.U.: Resources. V.C.O.: Funding acquisition, Writing—review & editing. The published version of the manuscript has been read and approved by all authors

Acknowledgments

Declared none

Conflicts of Interest

The authors of this manuscript declare that they have no conflicts of interest.

6. References

1. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*. 2011;7(3):270-279. doi: 10.1016/j.jalz.2011.03.008
2. Prajjwal P, Marsool MD, Inban P, Sharma B, Asharaf S, Aleti S, Gadam S, Al Sakini AS, Hadi DD. Vascular dementia subtypes, pathophysiology, genetics, neuroimaging, biomarkers, and treatment updates along with its association with Alzheimer's dementia and diabetes mellitus. *Disease-a-Month*. 2023;69(5):101557. doi:10.1016/j.disamonth.2023.101557
3. Jellinger KA, Korczyn AD. Are dementia with Lewy bodies and Parkinson's disease dementia the same disease?. *ASMS*. 2023; 14:489-524.
4. Ni'ma NS. The Bright Side and the Dark Side of Scopolamine (Pharmacology, Toxicology, and Clinical Use Review). *J Sci Technol Res Pharm*. 2022;2(1):18-25. doi:10.15294/jstrp.v2i6833
5. Amjad L, Shahrani M, Amini Khoei H. A Systematic Review of the Role of Gummosin in Improving Memory in the Scopolamine Impaired Memory Model. *Arch Razi Inst*. 2024;30;79(2):248. doi: 10.32592/ARI.2024.79.2.248
6. Aware CB, Patil DN, Suryawanshi SS, Mali PR, Rane MR, Gurav RG, Jadhav JP. Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *S. Afri J Bot*. 2022;151:512-528. doi: 10.1016/j.sajb.2022.05.028

7. Chaachouay N, Douira A, Zidane L. Herbal medicine used in the treatment of human diseases in the Rif, Northern Morocco. *Arab J Sci Eng.*. 2022;47(1):131-153.doi: 10.1007/s13369-021-05501-1

8. Writing Committee Members, Isselbacher EM, Preventza O, Hamilton Black III J, Augoustides JG, Beck AW, Bolen MA, Braverman AC, Bray BE, Brown-Zimmerman MM, Chen EP. 2022 ACC/AHA guideline for the diagnosis and management of aortic disease: a report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2022;80(24):e223-393. doi/full/10.1016/j.jacc.2022.08.004

9. Balkrishna A, Sharma N, Srivastava D, Kukreti A, Srivastava S, Arya V. Exploring the safety, efficacy, and bioactivity of herbal medicines: Bridging traditional wisdom and modern science in healthcare. *Future Integr Med*. 2024;3(1):35-49.**doi:** 10.14218/FIM.2023.00086

10. Sánchez M, González-Burgos E, Iglesias I, Lozano R, Gómez-Serranillos MP. Current uses and knowledge of medicinal plants in the Autonomous Community of Madrid (Spain): A descriptive cross-sectional study. BMC complementary medicine and therapies. 2020;20:1-3. doi:10.1186/s12906-020-03089-x

11. Gulukun EZ, Ari MM, Alu SE. Proximate composition and antinutritional factors of differently processed kidney bean (Phaseolus vulgaris) seeds. *Niger J Anim Prod.* 2020;47(5):169-175. doi: 10.51791/njap.v47i5.1274

12. Yanqiu H, Guojun F, Xiaoxu Y, Chang L, Dajun L, Zhishan Y. Phytohemagglutinin in Common Bean Germplasm Resources: Determination and Content Variation Analysis. *Chin Agric Sci Bull*. 2020;36(5):18-25. doi: 10.11924/j.issn.1000-6850.casb20190700421

13. Brunton LB, Lazio JS, Parker KL. Therapeutics. *The Pharmacological Basis of Therapeutics* [607-629]. New York: McGraw-Hill; 2005

14. Chase DL, Koelle MR. Biogenic amine neurotransmitters in C. elegans. WormBook: The Online Review of C. elegans Biology. 2007. Available at: https://www.ncbi.nlm.nih.gov/books/NBK19678/. [Accessed on April 16, 2025].

15. Osim EE, Erigbali P, Ofem EO, Okon UE. Chronic consumption of plantain (Musa paradisiaca) diet increases learning and memory in mice. *J Pharma Bio.* 2017;7:64-68.

16. Mosienko V, Bert B, Beis D, Matthes S, Fink H, Bader M, Alenina N. Exaggerated aggression and decreased anxiety in mice deficient in brain serotonin. *Transl psychiatry*. 2012;2(5):e122-.124

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

18. Podhorna J, Brown RE. 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.* 2002*;* 1:96-110.

19. Brown RE, Corey SC, Moore AK. Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. *Behavior genetics*. 1999;29:263-271.

20. Stahl SM, Mignon L, Meyer JM. Which comes first: atypical antipsychotic treatment or cardiometabolic risk?. *Acta Psychiatr Scand*. 2009;119(3):171-179. doi: 10.1111/j.1600-0447.2008.01334.x.

21. Guyton AC, Hall JE. Textbook of Medical Physiology (11th edition). Philadelphia: Elsevier Saunders. 2006

22. Livesey TR, Hullshof T, Howlett J. Glycaemic response and health-a systemic review and metaanalysis: relations between dietary glycaemic properties and health outcomes. *Am J Clin Nutr*, 2008; 87(1): 2585-2685.

23. Portas CM, Bjorvatn B, Ursin R. *Prog Neurobiol*, 2000; 60(1), 13-35.

24. Walther DJ, Peter JU, Winter S, Holtz M, Paulmann N, Grohmann M, Vowinctel J, Alamo BV, Wilhem CS, Ahnert HG, Bader M. Serotorylation of GTPases is a signal transduction pathway that triggers platel*et al*pha-granules release. *Cell*. 2003; 115(7), 851-862.

25. Ennaceur A, Delacour J. A new one trial test for neurobiological studies of memory in rats. *Neuroreport.* 1988*;* 8: 1173-1178.

26. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Processing.* 2012*;* 13(2): 93-110.

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

28. Bolivar VJ. Intra-session and inter-session habituation in mice: From inbred strain variability to linkage analysis. *Neurobiol Learn Mem.* 2010; 92(2): 200-214.

29. Blackford JU, Allen AH, Cowan RL, Avery SN. Amygdala and hippocampus fail to habituate to faces in individuals with an inhibited temperament. *Soc Cogn Affect Neurosci.* 2013*;* 8(2): 143-150.