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Short Communication

Anxiolytic and Antideppressive Effects of Cooked Beans (*Phaseolus Vulgaris*) and Serotonin Precursor Diets Following Scopolamine-Induced Mood Impairment in Cd1 Mice -

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ABSTRACT

The common African Bean (*Phaseolus vulgaris*) contains serotonin and its precursors among several other constituents. Serotonin is generally known to affect mood in humans. However, whether consumption of beans diet which contains serotonin will affect the mood notably, anxiety and depression in mice with scopolamine-induced mood impairment has not been previously ascertained. Therefore, the effects of consumption of cooked beans (*Phaseolus vulgaris*) as well as serotonin precursor diets on scopolamine-induced anxiety and depression in mice were studied. Sixty mice were randomly assigned into 6 groups namely; control, scopolamine only, 50% cooked beans diet + scopolamine, serotonin precursor (tryptophan) diet + scopolamine, 50% cooked beans diet only and serotonin precursor (tryptophan) diet only. The animals had access to clean water *ad libitum.* Light and dark box and the Elevated plus maze tests were used for evaluation of fear/anxiety. Forced swim test was used to assess for the depression in the animals. Results showed that the scopolamine only group had a significantly shortened light chamber duration compared to the control group ($p<0.05$). The light chamber duration of the other treated groups was significantly longer compared to the scopolamine only group. The open arm durations of the experimental diets treated groups were significantly longer compared to the scopolamine only group. The duration of immobility of the scopolamine only group was significantly increased compared to control and other groups that received cooked beans or serotonin precursor ($p<0.05$). In conclusion, consumption of beans and serotonin precursor diets alleviated anxiety and depression in scopolamine-induced anxiety and depression in mice. The anxiolytic and anti-depressive effects observed may be attributed to serotonin synthesized from tryptophan in beans.

Keywords: Beans; Anxiety; Serotonin; Scopolamine; Depression

INTRODUCTION

The common bean (*Phaseolus vulgaris)* is a dicotyledonous plant belonging to the pea family. It is a staple food for many people in different parts of the world [1]. It is a superb source of protein, carbohydrates, dietary fiber, 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 [1-3]. It has been reported that beans have anti-carcinogenic, anti-mutagenic [4], anti-inflammatory, anti-diabetic, hypoglycemic, cardio-protective and antioxidant effects [5]. It has also been reported that it contains serotonin and its precursor 5-Hydroxytrytophan (5-HTP) [6]. Beans contain other chemical compounds including saponins, tannins, glycosides, flavonoids etc. Aduema [7] reported that long term consumption of beans diet improves learning/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, and sleep [8]. 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 [9]. It is also reported to have direct influence on mood [10].

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 [11]. It is also used as anxiogenic agent for evaluation of anxiolytic effects of new drugs. Scopolamine has also been shown to impair mood in animals [12].

Owing to the adverse effects of synthetic drugs [13], 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 [14]. Therefore, natural products may provide a new source of beneficial neuropsychotropic drugs [13] provided they are scientifically validated and their mechanisms properly established.

Since beans contain serotonin that can potentially affect mood, it is, therefore, conceivable that the consumption of beans may affect moods such as anxiety and depressive strokes. Hence, this present study investigated the effects of consumption of common beans on anxiety and depression in mice with impaired mood, notably anxiety and depression using scopolamine.

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 ground 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 a uniform mixture.

Preparation of serotonin precursor diet: Serotonin precursor (5-Hydroxytryptophan) was obtained from Sigma Aldrich, Germany for use in this study. The estimation and preparation of the powdered 5-Hydroxytryptophan content of cooked beans was according to the method of Feldman and Lee [15] and modified by Mosienko *et al*., [16]. 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 given to the mice. An electric blender was used to blend the mixture to form the serotonin precursor diet.

Experimental animals and design

Sixty adults CD1 white mice weighing between 17 – 26 g obtained from the animal house of Physiology Department, University of Calabar, Nigeria were used for the study.

They were randomly assigned into 6 groups of 10 mice each, namely; control, scopolamine only, 50% cooked beans + scopolamine, serotonin precursor diet + scopolamine, 505 cooked beans only and 5HT precursor only groups respectively. The mice in the control group were fed normal rodent chow only and administered normal saline (1ml/kg bodyweight intraperitoneally). The second group of mice were fed normal rodent chow and administered Scopolamine

(1mg/kg bodyweight intraperitoneally). In the third group, the mice were fed cooked beans diet and administered scopolamine, while in the fourth group, mice were fed serotonin precursor diet and administered scopolamine. The fifth group comprised mice that were fed the cooked beans diet and administered with Scopolamine. The sixth group was fed serotonin precursor diet and administered normal saline. 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 maintained in a specific pathogen-free and well–ventilated housing unit at room temperature (28 + 2 °C) and humidity (85 + 5 ° per cent). The housing rooms were illuminated on a 12-hour light-dark cycle with clean drinking water *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

Light/Dark Transition Box test for anxiety: The light-dark transition box is a test of unconditioned anxiety and exploratory behaviour. It is based on the conflict between exploring in a novel environment and avoidance of bright light [17]. This box is divided into two compartments of unequal sizes. It is made up of plywood. The small compartment painted black has a measurement of 18 x 27 cm and constitutes 2/5 of the box. The larger (27 x 27 cm) compartment is painted white and makes up 3/5 of the box. Both compartments are linked by a door (7.5 x 7.5 cm) that is located at floor level in the centre of the wall separating the two compartments. The floor which is covered with Plexiglas is divided into 9 x 9 cm squares. The tests in this apparatus were conducted in a 2 x 5 m neurobehavioral laboratory which was lit by a 60 watts red lamp for background lighting. The mice were placed into the apparatus and allowed an exploration time of 5 minutes. The test sessions were recorded using video camera. Transitions, light chamber duration, stretch attend posture, rearing frequency, grooming duration were the behaviours scored. The test room was dimly lit while the bright light chamber was particularly lit by a small 2watt energy bulb.

Elevated plus maze test for anxiety: The Elevated plus Maze was built according to the description of Lister [18]. The maze has two open arms (45x5cm^2) with 0.25cm high edges and two closed arms (40x5cm^2) with 15cm high walls radiating from a central square (5x5cm). The open arms contain a slight ledge (4 mm high) to prevent the mice from slipping and falling off the edge. The closed arms provide a sense of safety because they are enclosed like most test of anxiety. This task exploits the conflict between the natural tendency of mice to explore the novel areas and fear of open spaces. The index of open arm avoidance also gives a measure of anxiety.

Prior to the test, the plus maze arms surfaces and closed sides cleaned with Methylated spirit to eliminate olfactory cures and to remove fecal boli and urine. Mice were placed in the central square of the plus maze such that the mice faced an open arm away from the experimenter upon placement. Immediately after placement, a stop watch was started and mice allowed exploring the apparatus for 5 minutes. The test sessions were recorded and video-taped. Increase in the time spent by the mice in the open arm as well as head dips indicate an antianxiety-like behaviour [19].

Forced swim test for depression: The forced swim test is a rodent behavioral test used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states. Mice were placed in an inescapable transparent tank that is filled with water and their escape related mobility behavior was measured. Successful implementation of the forced swim test requires adherence to certain procedural details and minimization of unwarranted stress to the mice [20]. The water tank was a cylindrical tank (30 cm height x 20 cm diameters) constructed of transparent Plexiglas. The water level was at 15 cm from the bottom and marked on the tank to ensure that the volume of water was consistent across mice used. The dimensions of the tanks were selected in a way that the mice were not able to touch the bottom of the tank, either with their feet or their tails, during the swimming test. The height of the tank was high enough to prevent the mice from escaping from the tank.

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. The analysis was done with an SPSS 18 statistical package. The mean values were considered significant at $p<0.05$.

RESULTS

Behaviours scored in light/dark transition box

Stretch attend posture (SAP): Figure 1 below shows the comparison of frequencies of SAP in the light and dark transition box test of the experimental animals. The SAP of the scopolamine only group was significantly increased compared to the control ($p<0.05$). The SAP of the other treated groups were significantly lower compared to the scopolamine only group ($p<0.05$).

Light chamber duration: While the scopolamine group was observed to have a significantly shortened light chamber duration compared to the control group ($p<0.05$), the light chamber duration

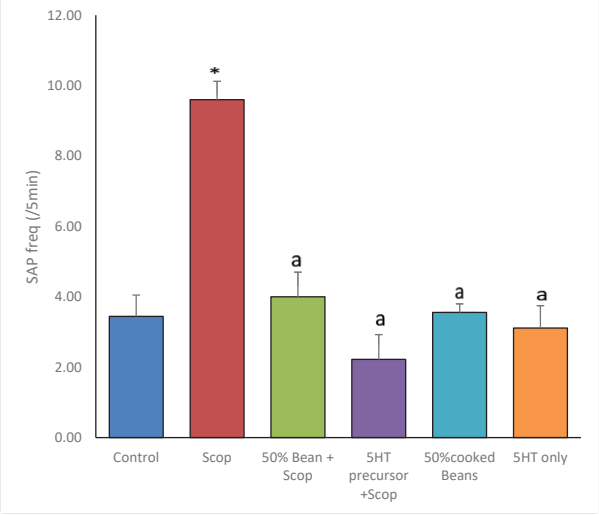

Figure 1: Comparison of the Stretch Attend Posture in the light and dark transition box test of the experimental animals.
Value are expressed as mean ± SEM, n = 10.
* = $p<0.05$ vs control;
a = $p<0.05$ vs scopolamine

of the other treated groups was significantly longer compared to the scopolamine group (Figure 2).

Dark chamber duration: The result showed that the dark chamber duration of the scopolamine only group was significantly longer ($p<0.05$) compared to the control group. The result also showed that the dark chamber duration of the 50% cooked beans diet + scopolamine group was significantly shorter compared to both the control and scopolamine only groups ($p<0.05$). While the dark chamber durations of the other treated groups were significantly shorter compared to the scopolamine only group, the values were significantly longer compared to the 50% cooked beans diet + scopolamine group ($p<0.05$) (Figure 3).

BEHAVIOURS SCORED IN THE ELEVATED PLUS MAZE

Frequency of open arm entries: In Figure 4, the result showed that the scopolamine group had a significantly lowered open arm entry compared to the control. The values for the other treated groups were significantly higher compared to the scopolamine group ($p<0.05$).

Duration of open arm entries: The duration of open arm stay of the scopolamine group was significantly shorter compared to the control ($p<0.05$). The values for the other treated groups were significantly longer compared to the scopolamine group ($p<0.05$). While the open arm durations of both the 5HT + scopolamine precursor and 5HT only groups were significantly shorter than that of the 50% cooked beans + scopolamine group ($p<0.05$), that of 5HT only group was significantly shorter than that of the 50% cooked beans group ($p<0.05$) (Figure 5).

Frequency of Head dips: The result in Figure 6 showed that the frequency of head dips for the scopolamine, 50% Beans + scopolamine and 5HT precursor + scopolamine groups were significantly lower compared to control ($p<0.05$). The values for 50% cooked Beans and 5HT groups were significantly higher compared to the control ($p<0.05$). The frequencies of head dips of the 50% Bean + scopolamine, 5HT precursor + scopolamine, 50% cooked Beans and 5HT only groups were significantly higher compared to the scopolamine only group ($p<0.05$). The values for 50% cooked Beans and 5HT only groups were significantly higher compared to both cooked beans + scopolamine and 5HT precursor + scopolamine groups ($p<0.05$).

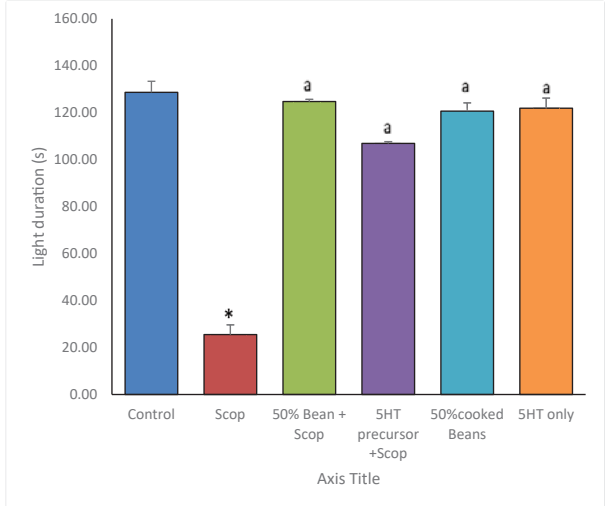

Figure 2: Comparison of light chamber duration in the light and dark transition box test of the experimental animals.
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine

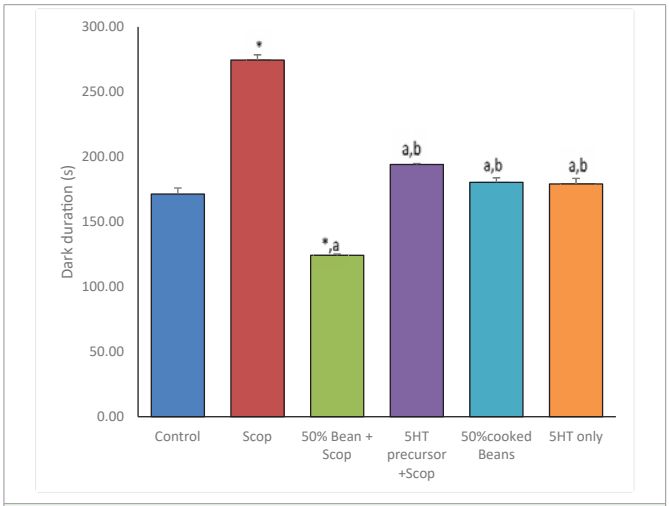

Figure 3: Comparison of dark chamber duration in the light and dark transition box test of the experimental animals.
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine
b = p<0.05 vs 50% cooked beans + scopolamine

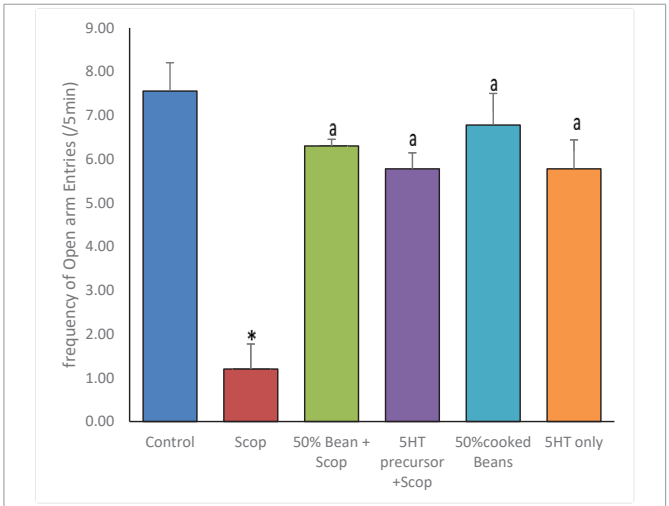

Figure 4: Comparison of frequency of open arm entries in elevated plus maze test of the experimental animals.
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control
a = p<0.05 vs scopolamine.

Forced Swim Test

Duration of Immobility in Forced swim test: Figure 7 the mean durations of immobility in force swim test of the experimental mice. The result showed that while the duration of immobility of the scopolamine only group was significantly increased compared to control ($p<0.05$), the values for other treated groups were significantly lower compared to the scopolamine only group.

DISCUSSION

The effects of consumption of cooked beans (*Phaseolus vulgaris*)

as well as serotonin precursor diets on scopolamine-impaired anxiety and depression in mice were studied.

In the light-dark transition box tests, most mice naturally demonstrate a preference for the dark chamber. Mice with less level of anxiety tend to venture more into the light compartment. The animals administered scopolamine only where observed to spend less time in the light chamber than they did in the dark chamber. This implied that scopolamine is anxiogenic. However, the animals fed the cooked beans or serotonin precursor diets were observed to spend more time in the light chamber and less in the dark chamber compared to scopolamine only treated mice. This implied that they had lower levels of anxiety. Furthermore, in the light and dark box environment, behaviours such as frequency of stretch attend posture in the light/dark chamber was observed to be less in groups of mice fed with cooked beans as well as serotonin precursor diets compared to scopolamine only treated group. This indicates the fact that there was a decreased level of anxiety in the group of mice fed with cooked beans as well as serotonin precursor diets.

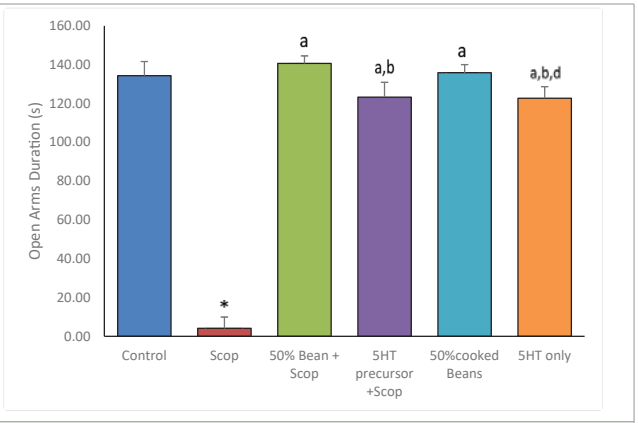

Figure 5: Comparison of open arm duration in elevated plus maze test of the experimental animals.
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine
b = p<0.05 vs 50% cooked beans + scopolamine
d = p<0.05 vs 50% cooked beans

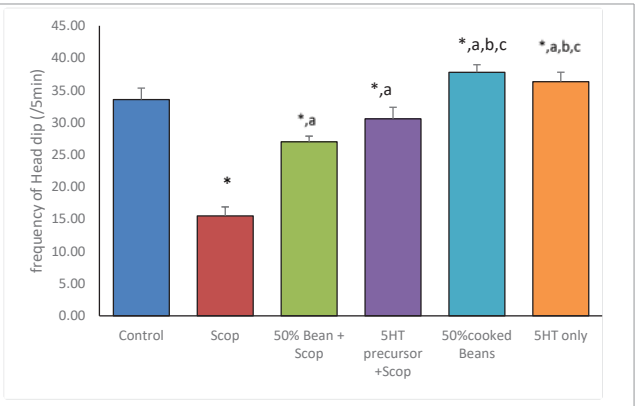

Figure 6: Comparison of head dips in elevated plus maze test of the experimental animals.
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine
b = p<0.05 vs 50% cooked beans + scopolamine
c = p<0.05 vs 5HT precursor + scopolamine

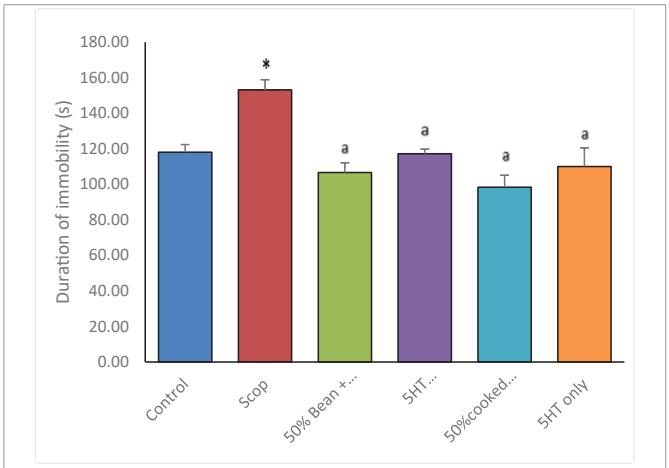

Figure 7: Comparison of duration of immobility in force swim test of the experimental mice.
Value are expressed as mean ± SEM, n = 10.
* = p<0.05 vs control;
a = p<0.05 vs scopolamine

Stretch attend posture is a risk assessment behaviour were rodents demonstrate forward elongation of the head and shoulder followed by retraction into its original position [21]. It is a behaviour exhibited by rodents introduced in a novel environment and it is a measure of anxiety. Fear and anxiety are basically controlled by neutral circuitry involving the amygdala mostly and the hypothalamus. Other areas of the brain that may be involved in the control of fear and anxiety are the nuclei of the hypothalamus. Electrical stimulation of the amygdala for instance, is associated with fear and feeling of terror in the animals [22]. Beans are known to contain cardiac glycosides and the neurotransmitter, serotonin, which reduce depressive feelings and promotes the relaxation of skeletal muscle tone [23]. Thus, it is possible that the presence of these compounds and other constituents in the beans could be responsible for the anxiolytic property of beans which act by inhibiting the excitability of the amygdala by increase in the threshold of response of the cells of these nuclei, thereby reducing fear related behaviour in the mice [23].

These observations can also be explained by the assertion of Young and Teff [24] that increased level of brain serotonin facilitates the calming, relaxing and mellowing serotonin neural circuits which frequently serve to counterbalance the arousing activating dopamine/noradrenaline circuit, so that anxious, agitated emotion occurs when a person's dopamine/noradrenaline activity arousal circuits are functioning strongly, without the calming, mellowing serotonin circuits functioning strongly as a compensatory counterbalance. It is possible that those mice did not show anxiety and fear related behaviours because cooked beans may have increased the level of brain serotonin.

The Elevated Plus Maze (EPM) consists of two 'open' arms and two closed 'arms' in the shape of a plus. The open arms are aversive to mice because they are open and the maze is elevated [18]. The closed arms provide a sense of safety because they are enclosed like most tests of anxiety (the light/dark box and open field). This task exploits the conflict between the natural tendency of mice to explore novel areas and fear of open spaces.

Behaviours such as open arm activity and head dipping are considered exploratory and a greater frequency of these measures shows a greater level of exploration. Fear behaviours usually include,

closed arm activity, stretch attend posture; a greater number of these measures implies a greater level of emotionality or fear [18]. Risk assessment behaviours such as head dips and stretch attend postures are indexes of levels of anxiety [25]. Another behaviour that strongly correlates with anxiety, is the closed arm duration in the elevated plus maze. Close arm duration was also found to be significantly longer in scopolamine only treated group compared to control. Fearful mice would normally spend more time in the closed arms of the elevated plus maze. This result shows that the scopolamine only treated mice showed increased level of fear and anxiety. On the other hand, the frequency of head dips for groups of mice fed with cooked beans as well as serotonin precursor diets were significantly higher compared to scopolamine only treated group. The open arms duration for groups of mice fed with cooked beans as well as serotonin precursor diets were also significantly higher compared to scopolamine only treated group. This showed a lower index level of anxiety and fear. Longer open arms duration and higher frequency of head dips are both behaviours which point to decreased anxiety. These behaviours correlate strongly, and the higher their values, the less the anxiety level. Therefore cooked beans consumption may be reducing anxiety in mice.

The forced swim test in mice was developed to test rodents for immobility because it was discovered that rodents became immobile after an initial swimming activity in an inescapable situation. The duration of immobility is considered a measure of despair or depression. The forced swim test result shows that the group of mice treated with scopolamine only had increased significant level of immobility compared to control and other experimental groups. The result of this work shows that, cooked beans as well as serotonin precursor diets improves depressive symptoms in mice models of depression. There are three parts of the brain that appear to play a role in depression: the hippocampus, amygdala, and prefrontal cortex.

Cooked beans have been shown to contain serotonin precursor (tryptophan) and antioxidants (flavonoids and polyphenols) [7] It is possible that cooked beans as well as serotonin precursor diets may have increased the biosynthesis of serotonin in the brain of the mice which could have caused reduced depressive activities in ours models [26]. Lee *et al.,* [27] reported that antioxidants improved stressful conditions in mice. The improved depressive conditions observed in this research work may be attributed to serotonin and antioxidants present in beans.

In conclusion, consumption of cooked beans and serotonin precursor diets displayed anxiolytic and anti-depressive potentials in animals fed such diets following scopolamine administration. These potentials may be attributed to serotonin and its precursors present in cooked beans diet.

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