Evaluating possible spatial memory enhancement and psychosomatic balance using *Daucus Carota* juice in wistar rats

ABSTRACT

Daucus carota (carrot) is one of the main vegetable crop cultivated worldwide. They are good sources of carbohydrates, minerals, dietary fiber, and phytochemicals like carotenes and phenolic compounds. In this study, the effect of carrot juice on memory impairment and anxiety-like behaviour was investigated. The study was carried out using memory and anxiety tasks such as passive avoidance test, navigation and elevated plus maze. This test involved three trials per week for a total period of three weeks. Twenty wistar rats of weight 120-150g were used. The animals were divided to four groups having five animals each group. Group one served as the control and was given feed and saline water. Group two was administered low dose of carrot juice (1ml/100g) orally. Group three was given an intermediate dose of 2ml/100g orally. The fourth group was administered a high dose (3ml/100g) of carrot juice orally. Elevated plus maze test was used to observe anxiety in the rat. Elevated plus maze usually used as a screening test for putative anxiolytic or anxiogenic compounds in neurobiological anxiety research. Navigation and passive avoidance test were used to test for memory impairment. Navigational maze is employed in behavioral neuroscience to study spatial stated that the test could be a very precise study of learning memory and spatial working and is also capable of accessing damages to cortical regions of the brain. The passive avoidance test is useful for evaluating the effect of novel chemicals entities on learning and memory as well as studying the mechanism involved in cognition. The study revealed that carrot juice has significant effects ($p<0.05$) in memory improvement but has no anxiolytic significance.

Keywords: *Daucus carota, wistar rats, Elevated Plus maze, Navigation maze, Passive avoidance test*

Introduction

The study of the brain's neurobiology has resulted in a common theoretical framework that spans molecular and cell biology on the one hand, and psychology and brain system biology on the other [1]. For decades, neuroscientists have been fascinated by the molecular and cellular basis of learning and memory. At the cellular and molecular level, the complexity of how we interpret, remember, and forget our experiences is impossible to comprehend. We're getting a better grasp of the chemical modifications that allow neurons to develop and store memories by using a variety of learning and memory paradigms in different model species. Memory is the retention and retrieval of events or facts composed of experiences, whereas learning is the incidence-dependent acquisition of skills and information [2]. Memory is measured by changes in an animal's behaviour after learning and reflects a variety of processes such as acquisition, consolidation, retention, retrieval, and performance [3]. Molecular mechanisms of memory have

focused mainly on the roadways that underlie acquisition. This emphasis is due, in large part, to the success of in vitro models of learning, including forms of synaptic plasticity such as long-term potentiation (LTP) [4] [5] Based on experimental and clinical evidences, acetylcholine (ACh) is considered the most important neurotransmitter involved in regulation of cognitive functions. [6] Alzheimer's disease (AD) is the most common agerelated neurodegenerative disorder characterized by cognitive dysfunction with memory impairment and behavioral disturbances [7]. Besides the neuropathological hallmarks of the disease, neurofibrillary tangles and neuritic plaques, AD is characterized by a consistent deficit in cholinergic neurotransmission particularly in basal forebrain [8].

According to Centre for Disease Control and Prevention memory decline has become a public health issue. It can have implication for living with and managing chronic disease, or performing everyday activities like cooking and cleaning. It is one of the earliest noticeable symptoms of Alzheimer's disease and related dementias [9]. Some memory decline can occur as adult age, but frequently forgetting how to perform routine tasks, for example, is not a normal part of aging and can affect a person's ability to live and function independently 10]. The research purpose and hypothesis will focus on evaluation of possible spatial memory enhancement and psychosomatic balancing activities of the plant .

Materials and Methods

Collection of Carrot

The carrot roots used were purchased from Aluu market, Aluu, River State. After a commercially available carrot has been washed with water, the carrot is peeled and cut into a 2 cm square dice, which is subjected to blanching till an internal temperature reaches 50 to 100° C. in water set to a temperature of 50 to 100° C. (for example, subjected to blanching till an internal temperature reaches 70° C. in water at a temperature of 70° C., the temperature at that time being 70° C., the same is true hereinafter), after which the carrot was crushed, and squeezed by filtration and being subjected to centrifugal separation (3000 rpm, 10 minutes) to provide the carrot juice.(according to modified method of Suzuki et al, 2002)

Collection of Experimental Animals

The experimental animals (Albino Wistar rat) used were collected from the animal house of the Physiology Department, in the University of Port Harcourt. The rats were kept to acclimatize for two (2) weeks. The rats were kept in cages with enough ventilation and sawdust was used to make beddings for them, they were fed with feed and water *ad libitum*.

Animal Grouping

The animals were grouped in four groups, with five animals in each group:

Group 1: Control, administered *ad libitum* water and feed

Group 2: 1ml/100g of carrot juice was administered to each animal in this group

Group 3: 2ml/100g of carrot juice was administered to each animal in this group

Group 4: 3ml/100g of carrot juice was administered to each animal in this group

Research Design

The carrot juice was administered orally, using a cannula during the period of acclimatization and in the course of the study.3 trials were done for each experimental group for 3 weeks duration.

Navigation Test

Overall, maze learning has been widely used to probe for the role of the hippocampus in rodent learning and memory. It's is a box with an entrance and exit, with a complicated maze. The animal is place at the entrance of the maze, and allowed to find its way to the exit within a five minute time frame.

Procedures of Navigation maze

- The navigation box is made of various chambers interconnected and interwoven to a final exit compartment.
- A trained mouse with normal neurologic condition will navigate to the last compartment within 5 minutes
- Those with seizure or memory impairment would not.

Procedure:

- A rat each was introduced into the navigating box test.
- Time taken (in 5 minutes) for each mouse to navigate from the origin to the last (exit) compartment was noted.

Passive Avoidance Test

This test is based on the innate aversion of rodents to brightly illuminated areas, and on the spontaneous exploratory behavior of rodents in response to mild stressors, ie, novel environment and light [11]. This model permits rat to freely explore two interconnected compartments that vary in size (2:1), color (white:black) and illumination (bright:dim). The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability. Its measures the basic ability to learn and remember the presence and place of a shock stimulation.

Individual rats are place in the light compartment, with the rat facing the door leading to the dark compartmentand observed in five minutes.

Elevated Plus Maze Test

The elevated plus maze is shaped like a plus sign, with two open elevated arms facing each other and separated by a central square, as well as two arms of the same size but contained by walls. The open arms of the maze are elevated off the ground, combining themes of unfamiliarity, openness, and elevation. The EPM is built on rodents' natural dislike to open places, and it employs a conflict between exploration and this aversion to achieve its goals [12] .Each rat was placed in the center of the plus-maze, facing one of the closed arms and observed for five minutes. The number of entries into the closed or the open arms, as well as the time spent in each type of arms was recorded. The entry with all four feet into one arm was defined as an arm entry. At the end of each trial the maze was wiped clean.

Statistical Analysis

Results of the study were presented as mean ± sem. Data were analyzed using ANOVA and Post Hoc LSD using SPSS version 23.

Result and Discussion

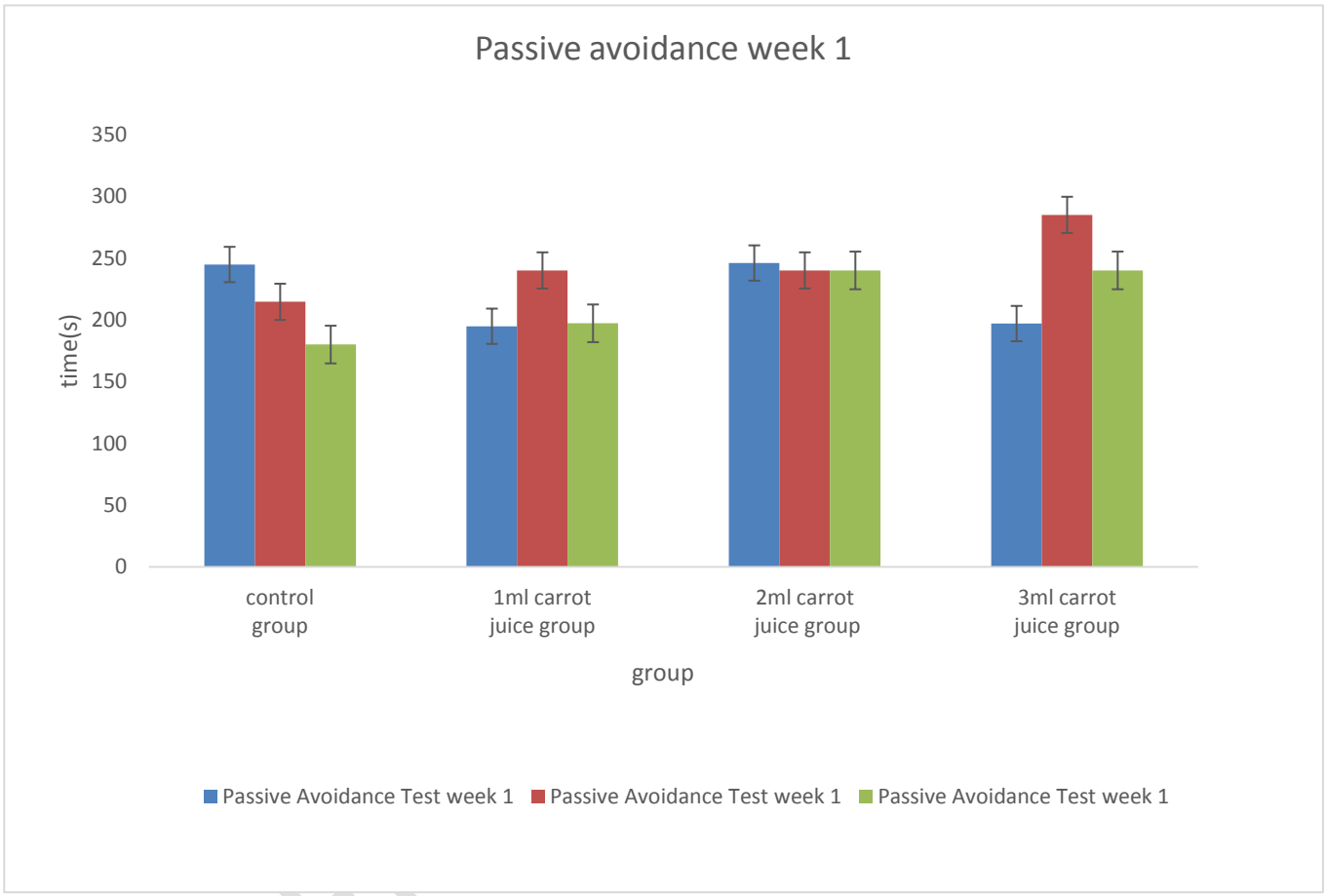

Figure 1 Spatial memory evaluation using passive avoidance test on the test groups' week 1

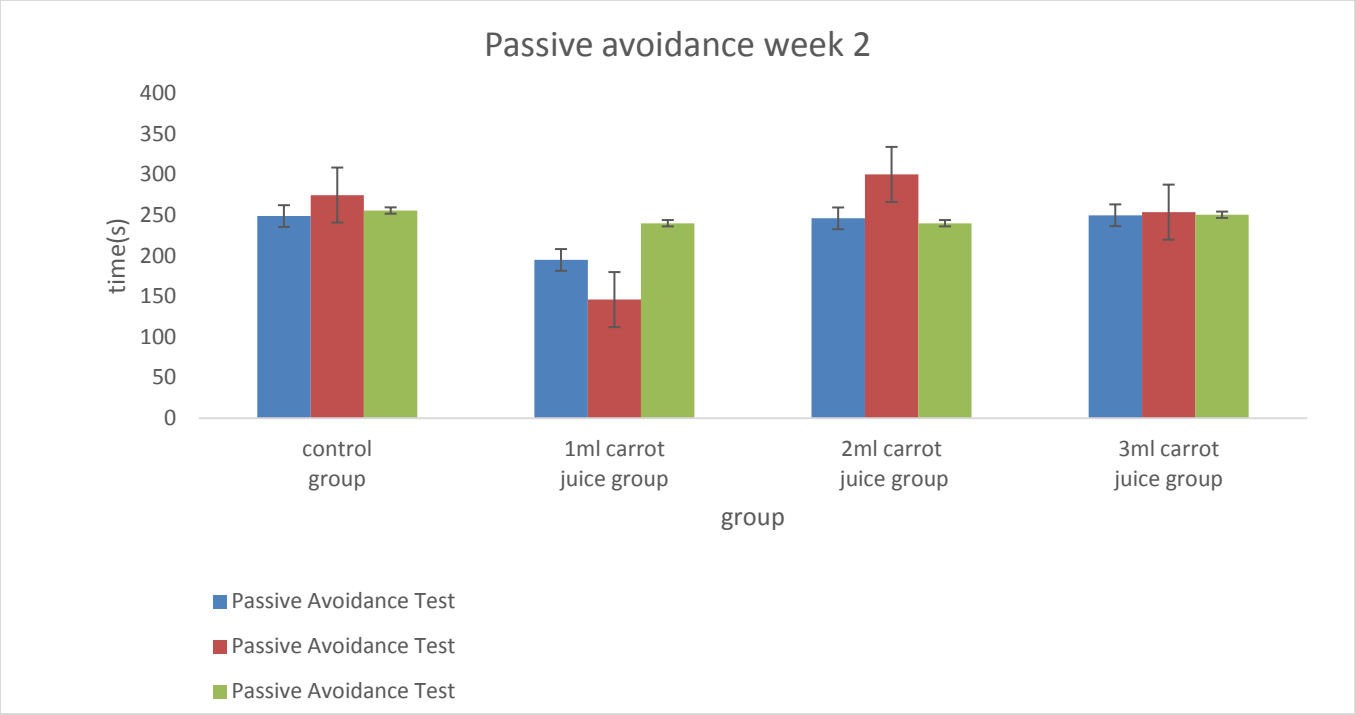

Figure 2 Spatial memory evaluation using passive avoidance test on the test groups' week 2

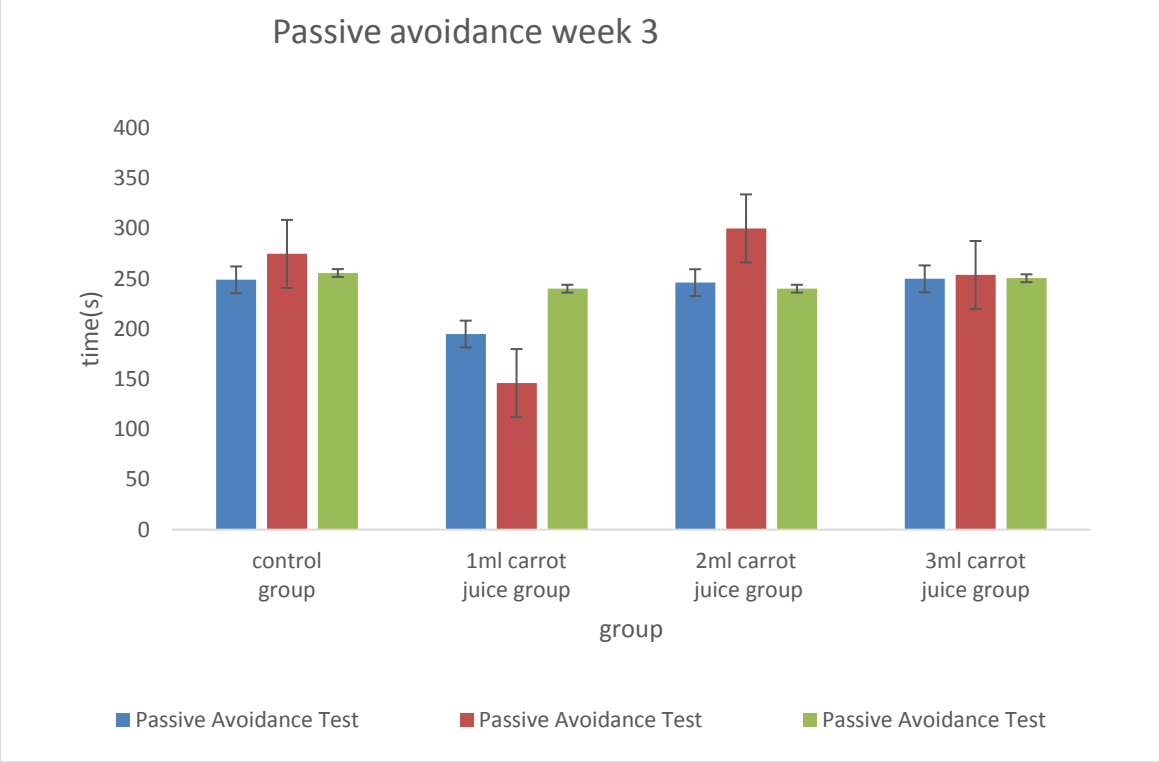

Figure 3 Spatial memory evaluation using passive avoidance test on the test groups' week 3

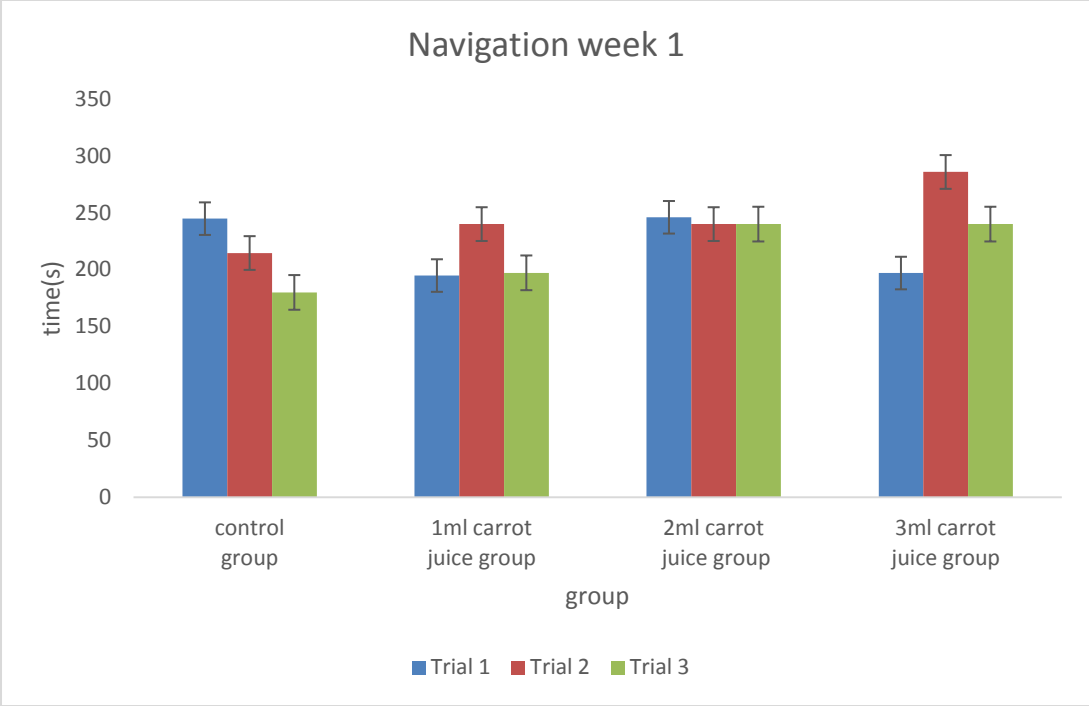

Figure 4 Evaluation of adaptive locomotion using Navigation maze Task in the test groups In week 1

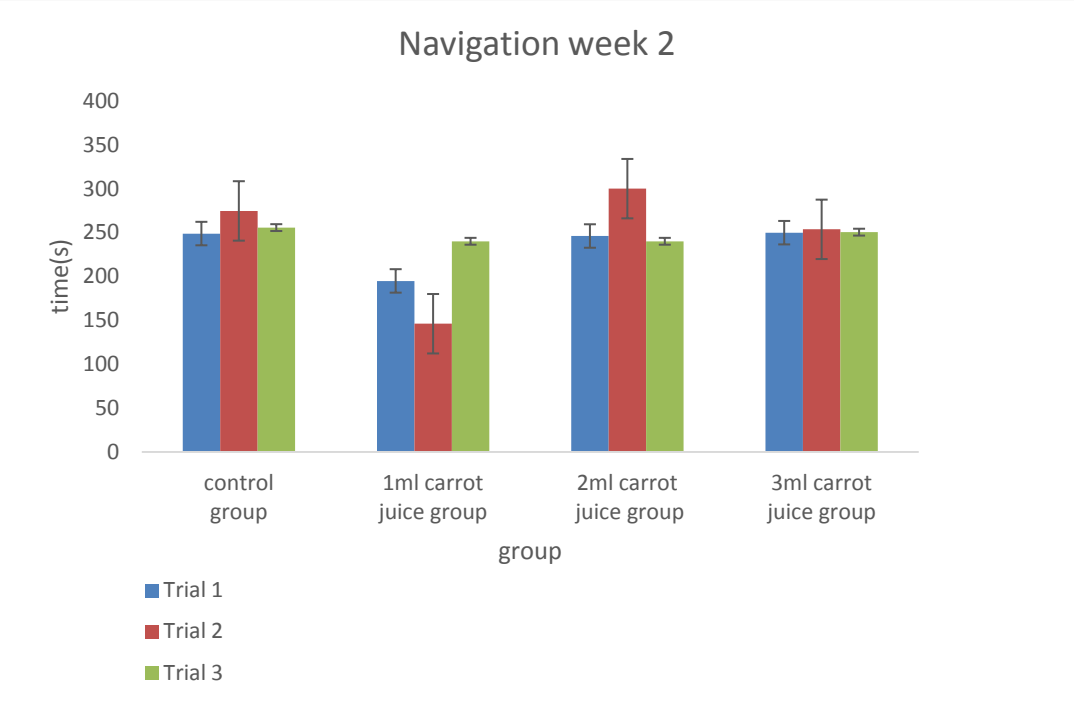

Figure 5 Evaluation of adaptive locomotion using Navigation maze Task in the test groups In week 2

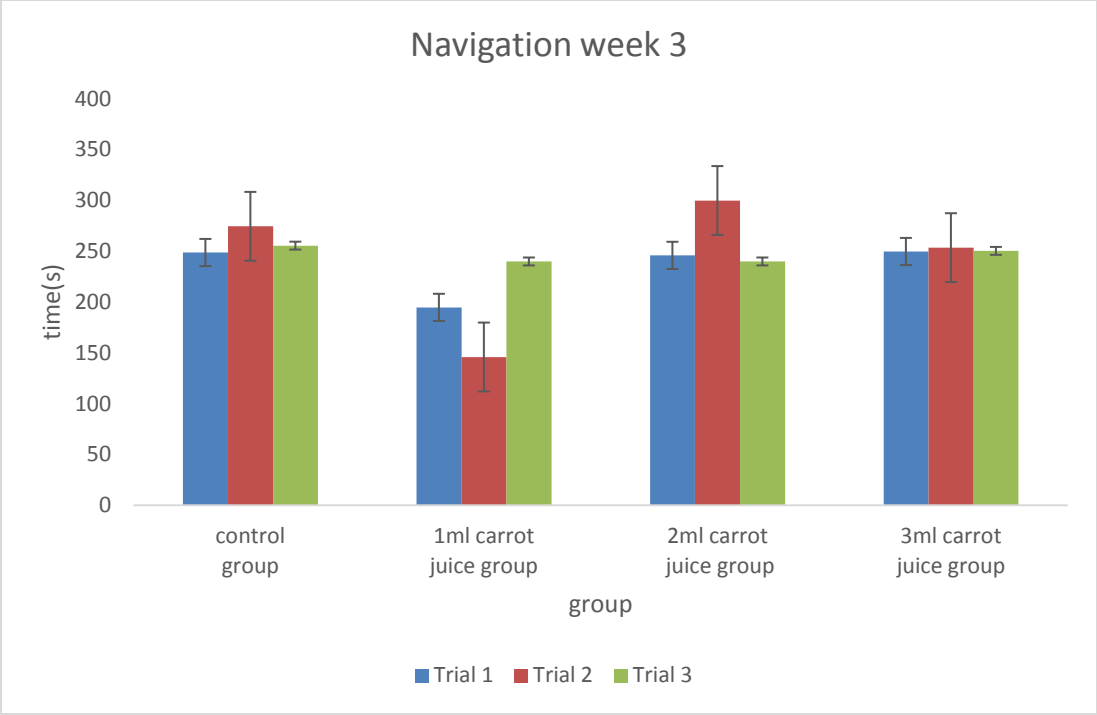

Figure 6 Evaluation of adaptive locomotion using Navigation maze Task in the test groups In week 3

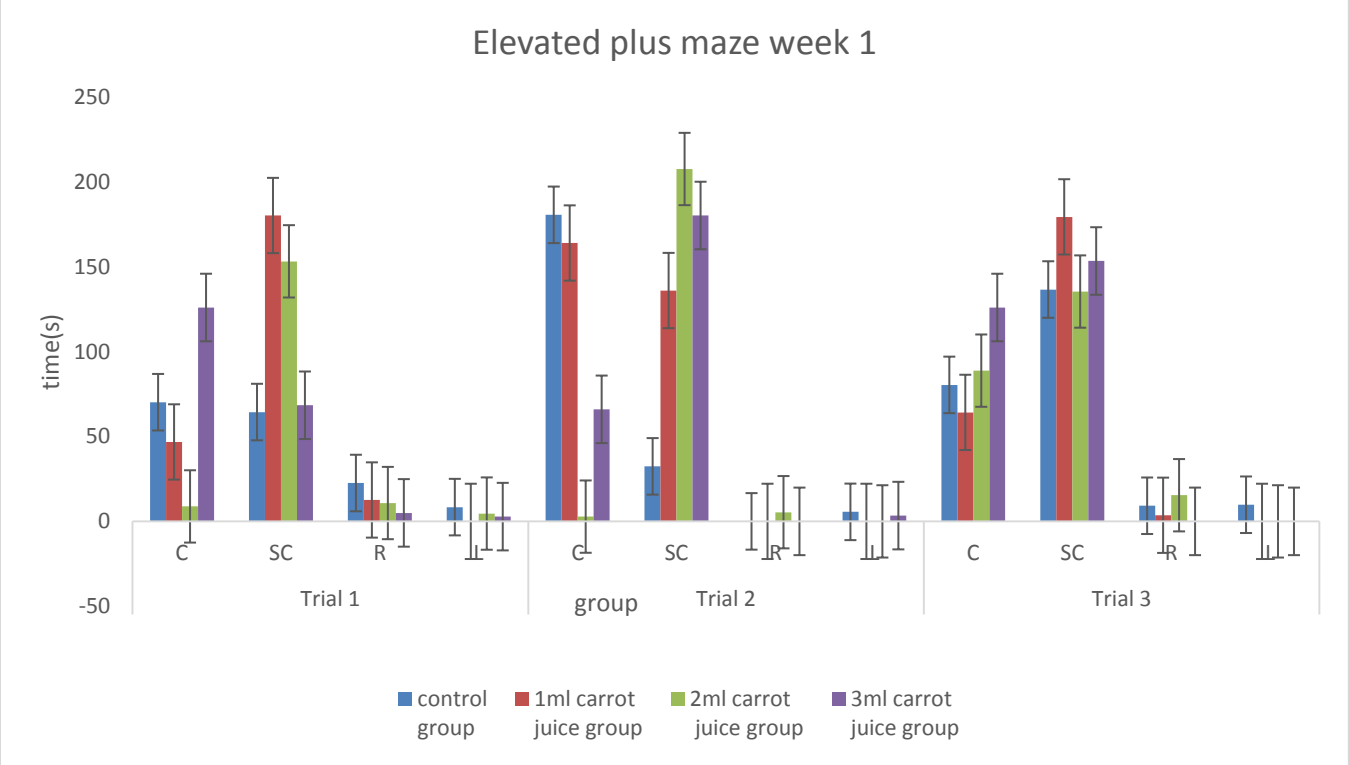

Figure 7 Evaluation of psychic response and anxiety using Elevated Plus Maze in the test and control group in week 1
(C= close arm, SC= semi close, R= right arm, L= left arm)

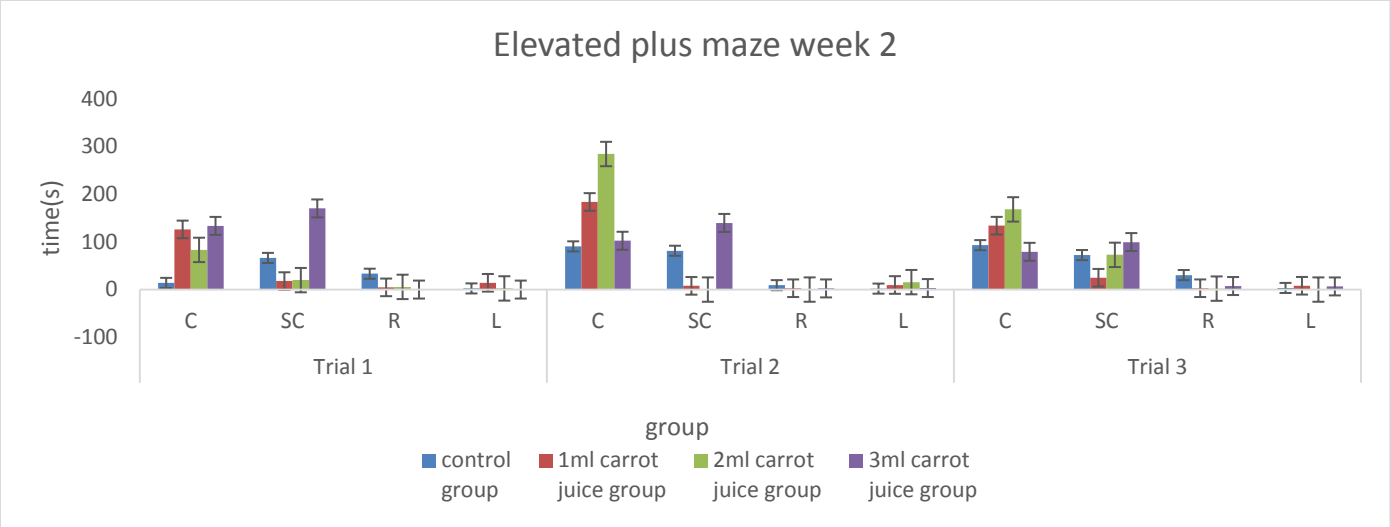

Figure 8 Evaluation of psychic response and anxiety using Elevated Plus Maze in the test and control group in week 2.
(C= close arm, SC= semi close, R= right arm, L= left arm)

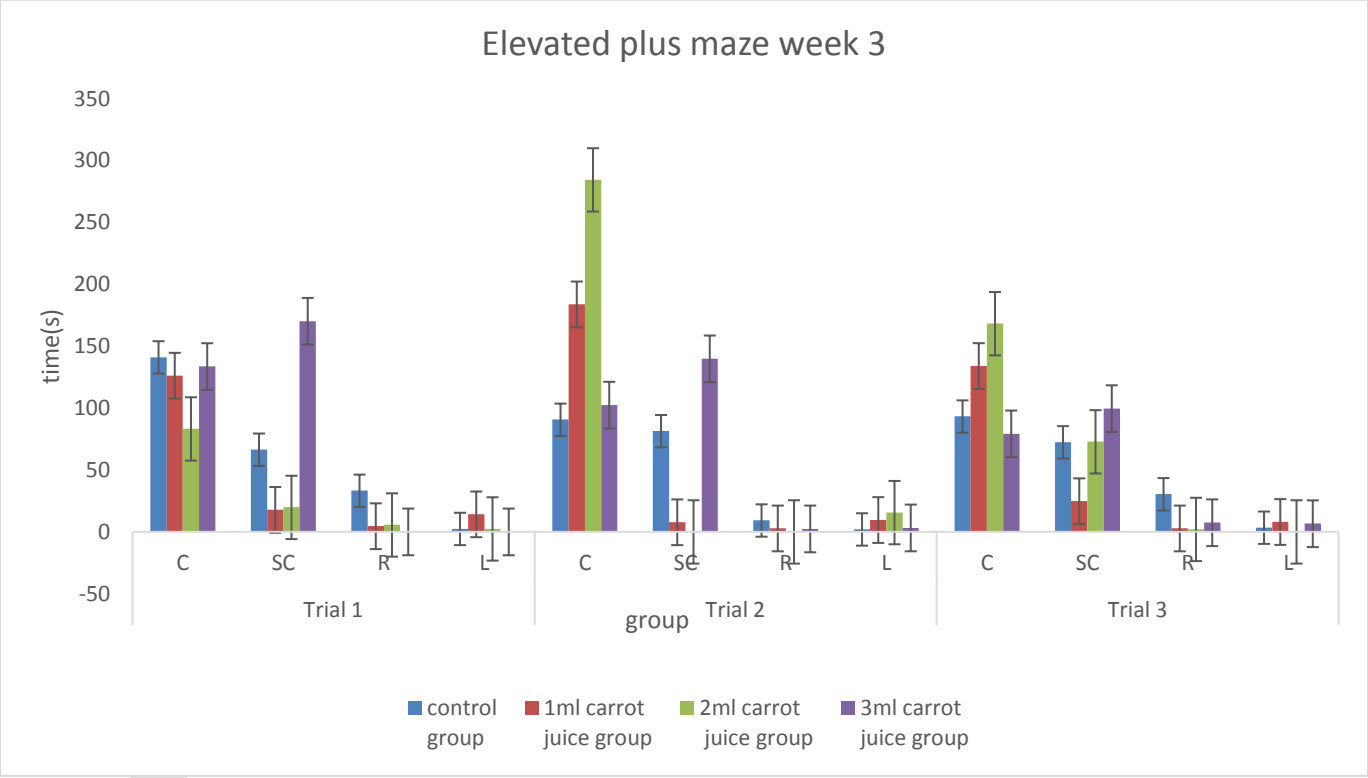

Figure 9 Evaluation of psychic response and anxiety using Elevated Plus Maze in the test and control group in week 3
(C= close arm, SC= semi close, R= right arm, L= left arm)

DISCUSSION

The present study was designed to evaluate the possible effect of carrot juice on memory enhancement or impairment and psychic behavior in albino wistar rats. The extrapolations from the study revealed that the juice could potentially be viable in spatial memory up regulation and enhanced psychic response as demonstrated in the study. The administration of the juice after the second week produced some levels of significance $p < 0.05$) in spatial tasks performance especially as observations progressed. The positive results obtained from the various maze tasks showed a dose-dependent and time-dependent evidence of the intrinsic potency of the carrot juice. Observations from the Passive Avoidance Task from week 1 (figures 1, 2, & 3) demonstrated that the juice could strengthen learning and enhance spatial memory For instance**,** the time taken for the animal to return to the dark box was significantly delayed as the dose treatment increased however**,** as expected, from figure 1 no significant effect was observed from week 1 to week 3 at control group across the whole trial in the study. Across the weeks of group two which was administered1ml of carrot juice, a difference was observed¸ though not significant. Group 3 which was administered 2ml of carrot juice showed significant ($p< 0.05$) difference. At 3ml carrot juice administered, significant difference could also be seen in this group. Overall, the result of the passive avoidance test showed that small quantity of carrot juice produce no significant different but with increase in dose administration, it produced a significant difference in most of the trials, showing an increment in memory function in terms of retention and retrieval. The quality of alertness and increase of the degree of awareness in the rats in the treated rat contributed immensely to their overall performance in the Passive Avoidance task and that accounted for enhanced spatial learning potential as measured by the total time spent in the light box after electrocution in the dark box. This observation agrees with previous similar study carrot out using carrot seed extract which improved memory [13].

Navigational maze is employed in behavioral neuroscience to study spatial stated that the test could be a very precise study of learning memory and spatial working and is also capable of accessing damages to cortical regions of the brain. Figures 4, 5, & 6 reported the results obtained for navigation test from week 1 to week 3. At control group, no significant effect was observed across the week and trials. At 1ml administration in group two, significant difference was recorded. Group three and four with treatment of 2ml and 3ml respectively, showed no difference. This study showed that with lesser dose of the treatment there was an improvement in memory, and higher doses did not make the memory any better, as observed in group three and four. This study agrees with similar study that where there was a reduced ability in the high dose group [14, 15]. Memory consolidation improved the adaptive locomotion in the test groups. There was active communication proceeding from the memory circuit towards the motor component of the body that enabled the animals to complete the process of navigation faster than the control group. The proprioceptive component of the peripheral nervous system that sub serves the cerebellum was well modulated and that accounted for the quality of motor performance observed in the test groups. Poverty of movement was adjudged to be very low in the groups that received the carrot juice. The general assessment of motor performance using the maze showed that the juice could be a good stimulator in certain aspects of motor functions. (16. 17 18, 19)

Elevated plus maze usually used as a screening test for putative anxiolytic or anxiogenic compounds in neurobiological anxiety research. (Figures 7, 8, & 9) reported the result obtained for elevated plus maze test from week 1 to week 3. The psychic behavior as exhibited by the treated rats showed slightly reduced anxiety like behavior as they were observed to spent more time in the open arm with explorative display of high quality though no consistency was

recorded for the three weeks of study. Generally, extrapolations from the study showed that carrot juice had no significant effect in ameliorating anxiety in albino wistar rat. This study agrees with similar study as rodents tend to remain on the open arms for less time compared with the closed arms [15, 20].

Conclusion

Carrot juice has demonstrated a significant effect on improving memory in wistar rat. The significant psychomotor effect as aided by increased efficiency in adaptive locomotion from the juice was observed to be useful especially in unfamiliar scenarios and seemingly complex tasks. The effective quantity of the juice has be commensurate to the level of mental task and challenges since the pattern of potency follows dose concentration and period of administration with significant spatial memory enhancement and psychosomatic balance

Ethical Approval

The animal handling, care and procedure were carried out on the specification of the University of Port Harcourt Ethical committee in line with the International Committee on Laboratory Animals.

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