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Neurobehavioural Studies on the Effects of Calcium Carbide on the Hippocampus of Adult Wistar Rats.

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ABSTRACT
The aim of this study is to investigate the neurobehavioural changes on the effects of calcium carbide on the hippocampus of adult wistar rats. Calcium carbide (CaC2) is a chemical compound used for a variety of industrial purposes especially in the production of acetylene gas. Itcontains impurities such as calcium phosphate and arsenate which when comes in contact with moisture produces phosphine and arsine both of which are toxic. Sixsty Wistar rats were grouped into 4 groups of 15 animals each. Group 1 were fed with standard rat chow and water. Group 2 were fed with standard rat chow, water and 2ml of naturally ripened banana juice. Group 3 was given 2ml of calcium carbide ripened banana juice orally and fed with standard rat chow and water. Group 4 were given 2ml of the calcium carbide treated banana and 200mg/kg body weight of vit C and fed with standard rat chow on daily basis. The rats in each group were subjected to the passive avoidance test, barnes maze test and Y-maze test which all are tools to test for memory and learning deficit in rodents. Statistical analysis was done using one-way Analysis of Variance. Results were compared using Post-Hoc test and considered significant at $p<0.05$. Passive avoidance test revealed that the rats showed impaired avoidance ability in the calcium carbide ripened banana group. Barnes maze study demonstrated that the rats showed impaired visual memory and cognition in the carbide treated group. In Y-maze test, the calcium carbide banana group showed decrease in the exploratory ability of the rats hence an impairment in working spatial memory. Calcium carbide causes impaired learning and memory ability in rats.

Keywords: Calcium carbide, passive avoidance test, barnes test, y-maze test, banana, learning and memory

INTRODUCTION
Fruits are a necessity to human health because it plays a role in supplying growth regulating factors which are essential to maintain a healthy life [7,33]. Fruit ripening normally is a natural phenomenon in which fruits undergo different physical and chemical changes [4,5,28]. Over time, various methods of artificial ripening has been developed to initiate ripening process in fruits during off season in order to meet up with the high demands of consumer. Various agents used in artificial ripening of fruits and vegetables include ethanol, methanol, methyl jasmonate, calcium carbide e.t.c. [7,11,28,33]. One of the cheapest and most commonly used of these ripening agent is the calcium carbide [4]. It reacts with water to form acetylene gas which induces the ripening in fruits. Calcium carbide contains traces of arsenic and phosphorous, toxins that are detrimental to the human health. Calcium carbide has properties of neurological and carcinogenic disorders [22] of which on short term basis can lead to dizziness, mood disturbanes, mental confusion, headache, cerebral edema and memory loss in long term basis. This prompted the need for food safety and to create an awareness on the risk related to consuming fruits infected by heavy metal or toxins [22].

The hippocampus is part of the limbic system, and plays a major role in the consolidation of information from short- term memory to long-term memory and in spatial memory that enables navigation.

The passive avoidance test is a fear – aggravated test used to evaluate learning and memory in rodent models with CNS disorders. The Y- maze test measures the willingness of rodents to explore new environments. The barnes maze test also assesses cognitive deficits in rodent models of CNS disorders.

This present study investigated the neurobehavioral changes on the effects of calcium carbide on the hippocampus of wistar rats.

MATERIALS AND METHODS
Animals: Sixty wistar rats weighing about 200-250g were used in this study. Animals were raised in the Animal House Unit in Faculty of Basic Medical Sciences, University of Port Harcourt. They were kept in clean plastic cages with stainless steel wire lids and fed on standard rat feed diet. Animlas were housed at a constant room temperature.

Procurement And Preparation Of Materials: Calcium Carbide was purchased from a welding workshop and matured unripe banana were purchased all from mile 3 market in Port Harcourt LGA of Rivers State, Nigeria. The matured unripe banana were divided into two equal parts. One part was allowed to ripen naturally at room temperature and the other part was ripened artificially with 5g of calcium carbide per 1Kg weight of banana as described by [19]. After ripening, 500g of each group of banana was blended with 500ml of distilled water using an electric blender and the juice extracted using a fine sieve which was then stored in a clean rubber bottle and stored in the refrigerator for further use [10].

Experimental Design: Rats were randomly distributed into four groups of fifteen rats each. Group 1 served as control and was given only distilled water. Group 2 were given 2ml of naturally ripened banana fruit and group 3 given 2ml of calcium carbide ripened banana fruit as described by [10]. Rats in group 4: were given 2ml of calcium carbide ripened banana fruit and 200mg of vitamin C per kg body weight dissolved in distilled water according to [25].

Passive Avoidance Test: The passive avoidance test box is made up of a light and a dark compartment demarcated by a small opening in between. The dark compartment had a mild electric foot shock and an over head lamp was placed in the light compartment. Each rats were allowed to explore both compartments and were given a mild foot shock once they entered the dark compartment. Their learning and memory ability were tested by placing them in the compartment with no electric shock which was the light compartment. The rats with normal learning and memory ability will avoid entering the compartment were they've been exposed to shock. The testing period was for 5 minutes (300 seconds) for each of the three trials. The recording was stopped if the rats re-enters the dark compartment or if it completed the testing period of 5 minutes.

Barnes Maze Test: This test is done on a circular platform with numerous escape holes ringed around the center of the platform. A target escape in form of a box is attached to one of the holes with an overhead bright lightening system which creates an aversive stimulus, encouraging the animal to seek out the target escape hole. Visual cues placed around the maze act as spatial cues. Each rat was placed in the center of the platform at each of the three trials and allowed to locate the target hole within 5 minutes. The experiment ends once the rat enters the target hole or once it runs out of the stipulated time.

Y Maze Test: Testing occurred in a Y-shaped maze with three plastic arms placed at a 120^0angle from each other. Each rat was introduced to the center of the maze, and allowed to explore all three arms for 5 minutes. An entry occurs when all four limbs are within the arm and the number of arm entries were recorded manually. Measure of spatial memory was defined as the entry into all three arms on consecutive choices.

Statistical Analysis: Findings were tabulated and analyzed with results expressed as mean ± SEM. Statistical analysis was done using one-way Analysis of Variance (ANOVA). The results were compared using Post-hoc (LSD) test. Results were considered significant at $p< 0.05$.

RESULTS

Table1: Estimation of degree of responsiveness in the control and test groups using Passive Avoidance Box

groups	treatment	Passive avoidance test (week 1)			Passive avoidance test (week 2)			Passive avoidance test (week 3)		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Group 1	Saline water	231.60 ± 27.92 b	300 .0 ± 0.0	300.0 ± 0.0 B	133.2 0 ± 68.09 a, b	196.4 0 ± 63.44 b, c	300.0 ± 0.0 b, c	290.4 0 ± 9.60 b, c	253. 8 ± 22.3 8 a, b, c	300. 0 ± 0.0 b, c
Group 2	Natural banana group	150.60 ± 60.99 b, c	300 .0 ± 0.0	300.0 ± 0.0 B	12.0 ± 1.22* c	279.2 ± 12.74 b,c	300.0 ± 0.0 b. c	277.2 ± 22.80 b, c	300. 0 ± 0.0* b,c	300. 0 ± 0.0* b,c
Group 3	Carbide + banana group	1.0 ± 0. 0 * a, c	1.0 ± 0.0	120.6 0 ± 73.23 * a, c	22.40 ± 2.69* c	35.80 ± 21.31 * a,	1.0 ± 0.0* a, c	62.80 ± 27.73 * a	8.60 ± 4.65 * a, c	36.6 0 ± 14.6 5 a, c
Group 4	Carbide + banana + vit C	300.0 ± 0.0 a, b	300 .0 ± 0.0	300.0 ± 0.0 b	192.6 ± 8.33 a, b	1.0 ± 0.0* a	180.4 0 ± 73.24 * a, b	60.8 ± 59.8* a	143. 6 ± 15.6 8* a, b	148. 2 ± 11.7 6* a, b

Values are presented as mean ± sem. N = 5.
* means values are statistically significant when compared to the control groups
"a" means values are statistically significant when compared to the natural banana group
"b" means values are statistically significant when compared to the calcium carbide + banana group
"c" means values are statistically significant when compared to the calcium carbide + banana + vit. C group

Table2: Visual memory and cognition assessment in the control and test groups using Barnes maze Task

Group	Treatment	Barnes maze Test (Week 1)			Barnes maze Test (week 2)			Barnes maze Test (week 3)		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Group 1	Control groups	183.20 ± 71.53 a, b	127.0 ± 25.72 a	85.60 ± 34.05 b	14.80 ± 1.96 b	46.40 ± 3.92 a, c	55.80 ± 2.94 a, b	47.20 ± 11.20 b, c	54.80 ± 16.71 b	51.80 ± 4.82 b
Group 2	Natural banana group	26.80 ± 1.96*c	11.0 ± 2.45* b	41.40 ± 0.25 b	21.20 ± 6.61 b	14.80 ± 2.94* b	14.60 ± 2.2* b, c	17.0 ± 5.39 b, c	13.60 ± 2.40 b, c	14.0± 2.16 b
Group 3	Carbide + banana group	34.0 ± 7.35* c	200.40 ± 60.99 a, c	232.6 0 ± 19.84 * a, c	148.2 0 ± 61.97 * a, c	44.0 ± 11.02 a, c	300.0 ± 0.0* a, c	216.20 ± 44.39* a	136.40 ± 30.02* a	243.4 0 ± 56.6* a, c
Group 4	Carbide+ banan+ Vit C	172.20 ± 52.17 a, b	45.80 ± 0.49 b	73.20 ± 0.49 b	31.40 ± 12.49 b	17.40 ± 2.21* b	58.80 ± 9.31 a, b	204.0± 58.79* a	78.80 ± 18.86 a	41.60 ± 7.10 b

Values are presented as mean ± sem. N=5.
* means values are statistically significant when compared to the control
"a" means values are statistically significant when compared to the natural banana group
"b" means values are statistically significant when compared to the calcium carbide + banana group
"c" means values are statistically significant when compared to the calcium carbide + banana + vit. C group

Table 3: Pattern path recognition memory test using Y-maze task in the control and test groups

Group	Treatment	Y-maze Test (Week 1)			Y-maze Test (week 2)			Y-maze Test (week 3)		
		base 1	Left arm 1	Right arm 1	base 2	Left arm 2	Right arm 2	base 3	Left arm 3	Right arm 3
Group 1	Control groups	14.20 ± 3.37 b	80.20 ± 42.46a, b,c	5.0 ± 2.45 c	13.0 ± 7.35	85.40 ± 46.44 c	58.60 ± 35.27	9.40 ± 2.91	19.60 ± 4.34	6.40 ± 5.40
Group 2	Natural banana group	5.80 ± 2.94 b	15.40 ± 3.63*	22.20 ± 7.70	9.80 ± 3.83	24.20 ± 9.21	8.60 ± 4.65	11.80 ± 1.56	8.0 ± 2.86	17.20 ± 0.92
Group 3	Carbide + banana group	123.40 ± 72.11* a,c	4.20 ± 1.96*	12.60 ± 7.10 c	59.40 ± 35.76	29.40 ± 12.32	99.0 ± 60.01	16.0 ± 11.14	25.80 ± 24.80	14.60 ± 5.74
Group 4	Carbide + banan+ Vit C	11.80 ± 6.61 b	1.0 ± 0.0*	91.40 ± 47.34 *b	18.20 ± 5.19	5.40 ± 2.69 *	19.80 ± 11.51	23.80 ± 3.69	5.40 ± 6.15	18.20 ± 5.19

Values are presented as mean ± sem. N=5.
* means values are statistically significant when compared to the control
"a" means values are statistically significant when compared to the natural banana group
"b" means values are statistically significant when compared to the calcium carbide + banana group
"c" means values are statistically significant when compared to the calcium carbide + banana + vit. C group

DISCUSSION

Passive avoidance test revealed that the rats showed impaired avoidance ability in the calcium carbide ripened banana group compared to the other groups. Phosphine a compound found in Calcium carbide causes agitation followed by convulsions, hyperactivity and lethargy in humans [16] and what has been referred to as nercosis or anaesthesia in animals [23]. The death of hippocampal cells as a result of necrosis, and the loss of painful sensation can hinder the creation, retention and retrieval of memory during the trial. This might be responsible for the poor performance of the rats administered with CaC2 during the passive avoidance test. Based on the works by [1,34], the above result could be said to be due to the disruption of astrocyte specific Na^+, K – ATPase or its provocation of electrical changes in the hippocampus and cerebral cortex which is similar to those noticed during generalized seizure. A similar result was also seen by [32] where they observed shorter lantencies to enter the shock compartment in the passive avoidance learning test and also by [29].

Barnes maze study demonstrated that the rats showed good visual memory and cognition in the group treated with naturally ripened banana compared to the other groups. This implies that naturally ripened banana increases the learning and memory capacities to form cognitive maps. This can be likened to the presence of potassium in bananas that function in generating electrical charges that helps the cells to function properly. Also, banana contains tryptophan an essential amino acid important for the production of serotonin which has a beneficial impact on learning and memory skills [7,13,20,24,31]. In the carbide treated banana group, it was observed that the rats were either reluctant or took longer time to perform a task as shown in the result. This is because calcium carbide has been proven to have very toxic effects such as mental confusion, mood disturbances, and sleepiness e.t.c [26]. It can also cause alterations in haematological and biochemical parameters [18].

In Y-maze test, the calcium carbide banana group showed significant decrease in the number of times the animals entered the arms. The decrease in the exploratory ability of the rats can be said to be as a result of the impairment in working spatial memory caused by calcium carbide. In a cadmium toxicity study for [15], a significant decrease in alteration behavior scores and a significant decrease in total number of times the animals entered the arms were observed in a Y-maze test that was done. [3] also noticed a decreased acquisition of avoidance behavior and alterations in an open field test caused by cadmium toxicity. The natural banana group showed a significant increase in the number of entries when compared to the calcium carbide ripened banana group.

Treatment with vitamin C exerts an antioxidant effect

and this may be responsible for the amelioration of calcium carbide induced toxicity. This ameliorative effect of vitamin C agrees with [17] where vitamin C was seen to ameliorate the effect on the spleen caused by methyl chloride toxicity and also by [2] where it showed a neuroprotective property against profenofos toxicity.

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