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Research Paper

Chronic Consumption of Calabash Chalk (NZU) Diet on Learning and Memory in Swiss White Mice

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SUMMARY: *The effect of chronic Consumption of calabash chalk onlearning and memory in the Swiss White Mice was carried out.A total of forty-five (45) healthy SwissWhiteMice (SWM) randomly divided into group 1, group11, and group 111 were carriedout.Gp1 used as the control was fed the normal rodent chow, GP11 was fed low dose (LD) while GP 111 received high dose (HD) of the Calabash Chalk extracts. Learning and memory test in these groups was carried out after two days of the feeding using the Moris Water Maize test((acquisition training, reversal training, probe trial and visible platform).The result showed thatin the acquisition training using the Morris water maize test, a significant difference was recorded among the three groups on the day 2 with GP1 having a latency period of 18.18 seconds while that of the LD and HD significantly increased to 45.00 and 55.87 seconds respectively .The increased was statistically significant (P< 0.01). In the reversal training of the Morris water maize test, the swim latency of both the LD and HD significantly increased (45.03 and 48.33 seconds respectively) compared to the GP1 (19.22 seconds) with p value <0.01. Probe trial showed a significantly lower quadrant duration of the LD and HD compared to the GP1 (p<0.05). With the visible platform test, swim latencies for LD and HD were (47.61±0.23 and 48.00±0.11 seconds respectively) significantly higher than that of the GP1 (20.00±0.07 seconds) with p value<0.01 .Calabash chalk may contribute to neurological enhancement, however cautious of the neurotoxic effects of its lead content following chronic consumption must be considered.*

Keywords*: Learning and memory; calabash chalk, Swiss White Mice.*

I. INTRODUCTION

Calabash Chalk is identified by different names such as Calabar Stone in English, La Craie or Argile by the French, Mabele Lingala in Congo, Nzu by the Igbo tribe of Nigeria, Ndom by the Efik/Ibibio of Nigeria. It is also known as Ebumba, Poto and Uloin other places (Abrahams et al 2013).

Geophagia is the practice of eating the earth, including soil and chalk. This practice is associated with religious beliefs, medication or as part of a regular diet (Dean et al 2007)). Eating Calabash Chalk is observed by both sexes and different age groups of people of African descent; there is the belief predominantly during pregnancy that it prevents vomiting, over-salivation and nausea (Abrahams et al 2013).Though native to Africa, Calabashchalk is now available in the United Kingdom (UK) in ethnic stores and markets(Ekong et al. 2012). In the UK, it is known to be associated with immigrants from south Asia (Abraham et al 2006), and West Africa (Dean et al 2006), with the latter consuming calabash chalk that has been imported from Nigeria and sold in ethnic shops.

The UK Food Standard Agency had reported presumably a total lead concentration in Calabash chalk to range from 8.2 mg/kg to 16.1 mg/kg, Kaolin being the underlying metal and organic pollutants in it (Dean et al 2004).The total lead concentrations are significantly greater than previous World Health Organization guideline limits of 1 mg Pb/kg in foodstuffs(). Authorities such as the American Academy of Pediatrics defined lead poisoning as blood levels higher than 10µg/dl (Barbosa, et al. 2005).

Lead poisoning causes a variety of symptoms and signs which vary depending on the individual and the duration of lead exposure (Karri et al 2008).The symptoms predominantly affect the central nervous system include insomnia, delirium, cognitive deficits, tremor, hallucinations and convulsions (Grant, 2009). Since Calabash chalk consumption is a common practice in Nigeria, with the reports that lead, one of its constituents lowers intelligent quotient in children and damages brain cells in mice (Ekong et al, 2014), it becomes necessary to find out possible effects on learning and memory using white Swiss mice as experimental models.

II. MATERIALS AND METHODS

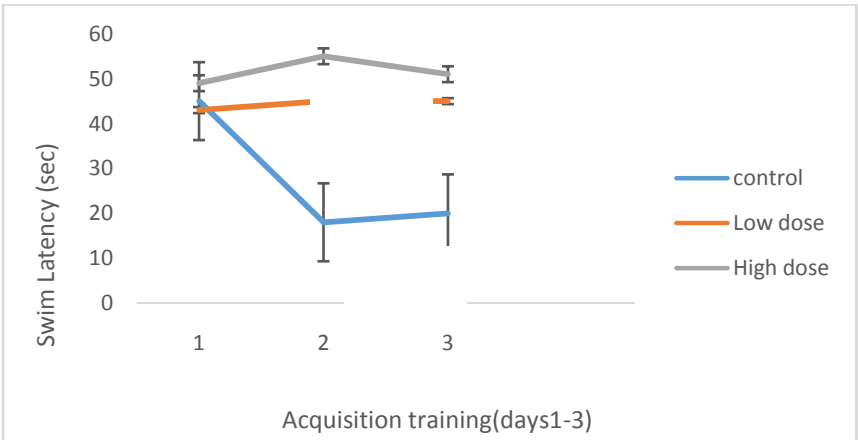
Subjects: A total of forty five Swiss white mice were procured from the Animal House of the Physiology Department of College of Medicine and Health sciences, Abia State University,Uturu,Nigeria. Proper animal acclimatization and home caging management was maintained with a well-designed feeding cages. Rat chow and calabash chalk were procured and adequate clean water made available for drinking and swimming. Also provided was the Morris water maze and liquid milk to make the water opaque. Activities of the animals were monitored with a digital video recording device.

Methods: The animals were randomly divided into group three groups of 15, group1, group11, and group 111 respectively.Gp1 used as the control was fed the normal rodent chow, water*ad libitum*. GP11 was fed low dose (LD) 10% of the extracts, while GP 111 received high dose (HD) 20% of the Calabash Chalk extracts. The feeding arrangement lasted for thirty days.

Tests for Learning and Memory: The Morris water maize was filled with water to a height of about 30cm and liquid milk added to make the water opaque. The water was allowed overnight in order to achieve room temperature. An escape plate was placed inside the water at a particular quadrant during each experiment; this was made hidden by the opaque water.The time it take a mouse to locate the escape hidden platform is called swim latency .Shorter the swim latency ,better the memory and learning. The test consisted of three days of acquisition training (when the animals were made to locate the escape platform)and 3 days of reversal training with the platform in the opposite quadrant with each mouse completing four trials per day and time taken to find and mount on the platform (the swim latency) recorded. On day 7, a probe trial with removal of the platform was done and (test of memory) duration of time each mouse spent in each quadrant (quadrant duration) recorded. On day 8, the milk colored- water was replaced by clear water making the platform visible and the procedure repeated and record taken.

III. RESULSTS

There was no significant difference in the learning ability of the three groups during the day 1 of the acquisition training in the Morris water maize test. A significant difference in swim latency was observed on day 2 among the three groups-.TheSwim latencies of 18.18, 45.00 and 55.87 seconds for the control,LD,HD groups respectively were recordedwhich differed significantly with P value <0.001amongst themselves. The swim latency of both the low and high dose groups on day three of the swim test were significantly longer ($p < 0.001$) compared to control. The reversal training from day 4 to day 6 showed a significant difference among the three groups with swim latency periods of 19.22 ,45.03,48.33 seconds being for the control ,LD ,HD group respectively and differed significantly at $p<0.001$. With the hidden plate form positioned in the north east during acquisition training and south west during the reversal training, the probe trial showed a significantly lower quadrant duration in the LD HD groups compared to the control at $P<0.001$. The swim latencies for control, LD, and HD groups during the visible platform test were 20.00±0.07, 47.61±0.23 and 48.00±0.11 seconds respectively; TheLD,and HD groups differing significantly ($p<0.001$) from the control group.



Acquisition training (days 1 – 3)

Figure I: Comparison of swim latency during acquisition training on days 1, 2 and 3 in the Morris water maze test.

Values are expressed as mean ± SEM, n = 10.

*significantly different from control at $p<0.001$; a = $p<0.001$ vs LD

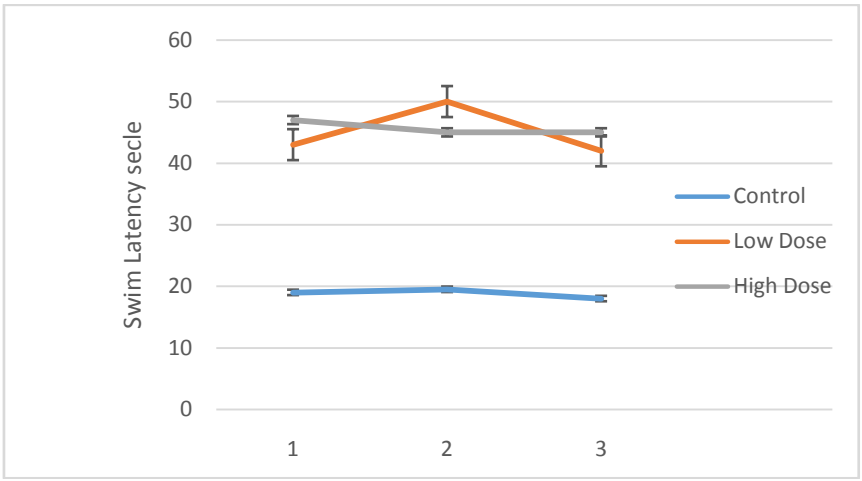


Figure 2: Comparison of swim latency during reversal training on day 4, 5 and 6.

Values are expressed as mean ± SEM. *p<0.001 vs control,a=P<0.05 vs low dose.

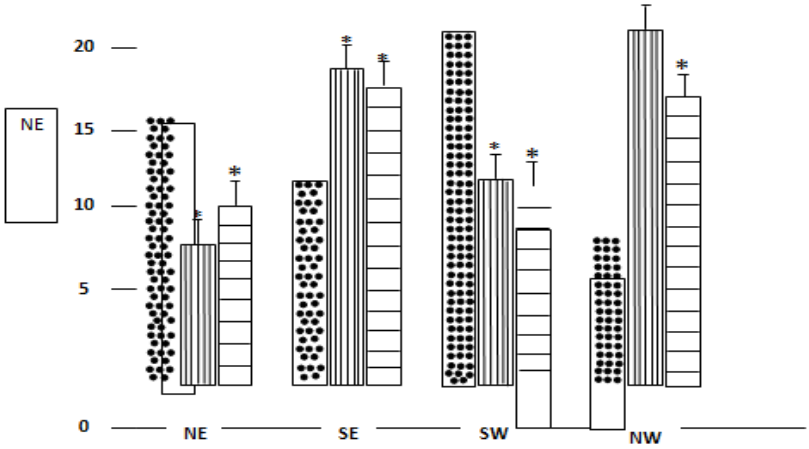


Figure 3: Comparison of swim latency during therobetrial o day 7.Values are expressed as mean ± SEM. *significantly different rom control

IV. DISCUSSIONS

The hidden platform is a test for visuo-spatial learning and memory powered by the hippocampus while the visible platform is a non-hippocampal task dependent on the dorsal striatum of the basal ganglia [McDonald & White 1994]. The hidden platform uses extra maze cues while the visible platform uses intra maze cues.

The swim latency is the time it took each mouse to locate and mount the escape platform. The shorter the swim latency, the better the learning process. Mice that learnt faster located the platform quicker than the others. Also, the steeper the swim latency gradient within the acquisition and reversal training, the better the learning curve and so the learning.

During the probe trail, when no escape platform was placed, the longer the time the mice stayed in the quadrants that had the escape platformplate form, the better the memory. So the mice that had better memory will be expected to spend more time in those quadrants where the escape platform was placed during the acquisition and reverse training. So the probe was to evaluate memory, while the acquisition and reversal training was for learning.

The result showed that the high dose group were the worst learners followed by the low dose group. So their learning ability was negatively affected by calabash chalk consumption as the control group learnt and did better from the second day of acquisition till the last day of reversal training, while the test groups improved only little in their learning. Remembering that the escape platform was placed in the North East and South West locations during the acquisition and reversal training respectively, any mouse that learnt well during those six days will spend more time in those two quadrants as the control group did during the probe trail test. This shows

that the mouse was able to remember their locations thus indicating better memory. While the control group were spending time in the quadrants that had escape platform (during the acquisition and reversal training), the test groups were wasting time exploring the quadrates that had no escape platform during the acquisition & reversal trainings. The swim latencies during the visible platform for the high dose and low dose groups were significantly higher than the control thus indicating that the test groups had lower visually assisted learning using the intra maze cues.

Our findings corroborate the report of Canfield et al. [2003] thathigh blood lead concentration was associated with low IQ in children, and that any substance containing lead damages the brain in rat fetuses and could have a negative effect on learning and memory (Ekong et al. [2014].Cautions should therefore be exercised by pregnant women and school children who are in the habit of snacking calabashchalk.

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