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Effects of Exposure of Hippocampus in Adult Wistar Rats (*Rattus norvegicus*) to hyoscyamine Fraction of *Datura stramonium* Seeds at Adolescence

Idris Abdu Tela[1*], Sunday Abraham Musa[2], Ibrahim Abdullahi Iliya[3], James Oliver Nzalak[4]

[1]Department of Anatomy,
Faculty of Basic Medical Sciences,
College of Health Science,
Bayero University Kano, Nigeria.

[2]Department of Human Anatomy,
Faculty of Basic Medical Sciences,
College of Medical Science,
Ahmadu Bello University Zaria,
Kaduna State, Nigeria.

[3]Department of Human Anatomy,
Faculty of Basic Medical Sciences,
College of Medical Science,
Federal University, Dutse,
Jigawa State, Nigeria.

[4]Department of Veterinary Anatomy,
Faculty of Veterinary Medicine,
Ahmadu Bello University Zaria,
Kaduna State, Nigeria.

Email: gwtel4u@gmail.com

Abstract

Adolescence is a critical period of development in mammals characterized by risk-taking and adventures. The study was designed to evaluate the effects of exposure to hyoscyamine fraction of Daturastramonium seeds at adolescence on the hippocampus of adult Wistar rats. Fresh seeds of D.stramonium were procured, macerated and fractionated using high-performance liquid chromatography (HPLC). Twelve (12) adolescent Wistar rats with an average weight of 150±0.02 grams of equal gender were used for the study. Equivalent body weight of normal saline and 800 mg/kgbwt of hyoscyamine fraction of D.stramonium were orally administered for three weeks, from postnatal days (PND) 21-42. At adulthood, the animals were tested for memory test using Morris water maze (MWM) and Novel object recognition test (NORT) paradigms. The data acquired were

**Author for Correspondence*

expressed as mean ± SEM. Student's-t-test and repeated measures ANOVA with Fisher's multiple comparisons post-hoc tests were used to obtain mean differences using Minitab 17 (LLC., U.K.) statistical package software. P< 0.05 were considered statistically significant. Significant difference in the exploration time (p = 0.047), but nostatistically significant difference was observed (p = 0.648) in the Morris water maze test between groups. The CA1 region of the treated group showed significant cytoplasmic vacuolations, hyperchromasia and necrosis. In conclusion, adolescent exposure to hyoscyamine fraction of D. stramonium induces impairment of hippocampal cognitive function which may predisposes progression of neurodegenerative diseases at adulthood.

Keywords: Adolescence, fractionation, hippocampus, hyoscyamine, memory

Introduction

Adolescence is a period of transition of physical and psychological development (Christie & Viner, 2005), and it heralded the vulnerability of stress and in some situations, opportunities to alleviate adverse effects of events experienced earlier during developmental processes (Dahl, 2004). In rats, the period lasts from the postnatal day (PND) 21 following birth until 60 days of age (Tirelli *et al*., 2003; Adriani & Laviola, 2004). It is marked by significant hormonal and behavioural changes, as well as substantial alterations in structural and neurobiological remodelling, which occur together in the cortical and limbic circuits (Romeo *et al*., 2002). This change leads to cognitive, emotional, social, and sexual maturation (Spear, 2000; Sisk &Zehr, 2005). The period of adolescence can be subdivided into three intervals, early- (pre-pubescent, PND 22-34), mid- (pubescent, PND 35-47) and late adolescence (sexually matured, PND 48-60) (Laviola *et al*., 2003; Adriani *et al*., 2004). In addition to increased open field activity (Hefner and Holmes, 2007; Moore *et al*., 2011; Blaney *et al*., 2013; Molenhuis *et al*., 2014), increased food consumption which is accompanied by high energy expenditure (Moore *et al*., 2011) throughout all three stages of adolescence, studies have reported significant developmental changes also in the medial temporal lobe, specifically the hippocampus, which is responsible for learning and memory (Spear, 2000; Paus, 2005; Mills *et al*., 2014). Furthermore, it was demonstrated that adolescent mice (PND 26-28) often exhibit a highly flexible behaviour compared to adults (PND 60-70) in a multiple-choice reversal learning test (Johnson & Wilbrecht, 2011). Other changes reported include increased risk-taking (Laviola*et al*., 2003) and sensitivity to drugs and alcohol consumption (Adriani *et al*., 2004; Hefner and Holmes, 2007).

The hippocampus is essential for the formation and retrieval of episodic and contextual memories in humans and animals. It is well established that that hippocampus is involved in the spatial layout and structural representation of an environment in both rodents and that cell loss or dysfunction in this area produces profound amnesia for newly acquired information (Anagnostaras *et al*., 1999; Squire *et al*., 2004; Frankland & Bontempi, 2005; Moscovitch *et al*., 2006; Wiltgen *et al*., 2006) and humans (Maguire *et al*., 1999, 2000) and also serves spatial memory processing (Guderian *et al*., 2015).

Drug abuse is a major public health problem all over the world (UNODC, 2010). The use and abuse of drugs by adolescents have become one of the most disturbing health related phenomena in Nigeria and other parts of the world (NDLEA, 1997), and this have also been linked to progression of neurodegenerative disorders during adulthood (Nestler & Malenka, 2004). *Datura stramonium* contains tropane alkaloids induces hallucinations and have also been implicated in the disorders of hippocampal development in rats (Ishola & Adeniyi, 2013).

The aim of the present study was to evaluate whether exposure to hyoscyamine fraction of *D. stramonium* at adolescence induces hippocampus damage and memory loss in adult Wistar Rats. The study may provide awareness to the government agencies such as National Agency for Food and Drug Administration and Control (NAFDAC) and National Drugs Law Enforcement Agency (NDLEA) to beam their searchlights beyond control and inhibition of the use of conventional drugs of abuse to also ethnomedicinal plants of abuse such as *D. stramonium* seeds.

Materials and Methods

Collection of plant materials, extraction and fractionation

Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2018/042). A fresh *D. stramonium* seed was procured from Sharada residential area of Kano Municipal Local Government, Kano State, Nigeria. The seeds were identified and a voucher number (VN108) was issued at the herbarium of the Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The seeds were separated from the pods, washed thoroughly with clean tap water and air-dried under shade. Two thousand grams of the dried seeds were weighed using a digital weighing machine, grounded to a pulp using an electronic blender. The pulverized seeds powder was cold macerated with 70% ethanol and partition-fractioned with A 5 ml portion of 10% sulphuric acid (H_2SO_4) using a modified Kamada *et al* (1986) and Djilani *et al*., (2006). The quantity of the fraction was obtained by the high-performance liquid chromatography (HPLC). Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

Plate I: *D. stramonium* seeds plant (**A**), fruits(**B**), dried seeds (**C**)

Experimental Animals and Design

Twelve (12) apparently healthy adolescent Wistar rats at postnatal day (PND) 21-42 comprised of equal genders were procured from the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, Bayero University Kano. The animals were housed and allowed to acclimatize for two weeks at ambient temperature, with alternate day and night cycles natural condition. Rat chow (*Vital feeds*®) and tap water were made available to the animal's *ad libitum*. The animals were randomly selected and divided into two groups. The first group received a single daily dose of equivalent body weight of normal saline while the treated group received 800 mg/kgbwt of hyoscyamine fraction of *D. stramonium* seeds orally for three weeks from (PND) 21-42. After the treatment, the animals were monitored to reach adulthood (PND) 60 – 75.

Novel object recognition test

The aim was to test short-term memory after administration of the hyosyamine fraction of the *D. stramonium* seeds. The test consisted of three phases i.e. (habituation, sampling and test) which was completed in two days. For each phase of this test, the open field arena was thoroughly cleaned with an unscented bleach germicidal wipe, 70% Ethanol followed by distilled water before initial use. A day before object exposure, the rats were habituated to the open field arena in a 50 x 50 cm wooden box. Before habituation session, a SONY® (Model DCR – PJ5E) digital and a video camera was used for a proper video covering of the rats in the maze. A rat at a time was gently removed from the home cage and placed in the centre of the arena. The video covering system was turned on and the rat was allowed to freely explore the arena for 10 minutes. At the end of every session, the arena thoroughly sanitized before next session began. This was repeated for all the rats until all got habituated the arena. The same protocol was observed during the sampling and test phases only that, two identical objects (A_1 and A_2) were used and two unidentical objects (A and B) objects for were used 15 minutes respectively.

Morris water maze

The aim was to test spatial learning and memory ability. This was carried out for six consecutive days using a modified Morris method (1984). The apparatus consisted of a circular Aluminium tank of 100 cm diameter and 60 cm depth with an escape platform of 20 cm long and 12 cm diameter, filled with a pool of clean water of about two-third of the tank at 22 – 25^{o}C, deep enough to expose 2.54 cm (1 inch) of the platform above the water surface. A digital video device, SONY® (Model DCR – PJ5E) was suspended directly over the pool to capture the entire setup. The rats were trained for 5 days with a methylene-blue coloured water that submerged the platform about 1 inch under. A latency period of 60 sec was allowed for each to find the platform. This was repeated for all the rats at five different locations by changing the positions of the platform in the pool within the N, E, S, and W directions following Qing *et al*.(2008) protocol. The same protocol was observed during the test day 6, however, 30 second for trial and the escape platform was removed. The time taken for each rat to identify the usual position of the platform was recorded and while all videos recorded for the trials were analyzed for the escape latency.

Animal Sacrifice and Histological Methodology

The animals were euthanized using 75% Ketamine (10 mg/ml USP) anaesthesia, the brains were dissected, removed and preserved in Bouin's fluid for histological procedures. The tissues were processed in the Department of Pathology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, Kaduna state, Nigeria. The brain tissues were dehydrated in different grades of alcohol and cleared in xylene using an automatic processing machine

(Shandon Southern Duplex Processor). The tissues were then infiltrated with paraffin wax and blocked in the coronal plane. Serial sections of the blocks were taken at 8 µm with a (LeitzWetzlar) microtome, mounted on glass slides and allowed to dry overnight. The staining technique employed was hematoxylin and eosin in paraffin sections (Lillie, 1965). Sections were then observed under a light Olympus Binocular Microscope (Ch-20i, Uttar Pradesh, India) high magnifications (x 40) and micrographs were taken with the help of *Celestron*® eyepiece digital camera (EC 3.0 MP, China). Coronal sections of the hippocampus were observed in the treated rats and compared to the controls.

Statistical Analyses

The data were expressed as mean ± SEM. Independent two samples t-test and pairwise General Linear Model (GLM) of repeated measure ANOVA followed with Fisher's multiple comparisons post-hoc was carried out to find the mean differences in the escape latency, exploration, discrimination and novelty preference time between groups using *Minitab* 17 (LLC., U.K.) statistical package software. $P< 0.05$ were considered statistically significant. All figures and charts were constructed using *GraphPad* Prism 8.

Results

Figure 1 (above)shows the result of the test-phase in novel object recognition test experiment between the control and adolescent Wistar rats treated with a multiple dose of 800 mg/kgbwt hyoscyamine fraction of *D. stramonium* groups using two non-identical objects; a previously known object (A) and unknown (B) object. The control group spent a significant amount of time exploring the non-familiar (B) object ($p = 0.047$) than the time taken to explore the familiar (A) one. However, the treated group showed no statistically significant difference ($p = 0.845$) in the exploration time between the objects.

Figure 1 (below) shows Morris water maze test in Wistar rats treated with equivalent body weight of normal saline and 800 mg/kgbwt hyoscyamine fraction of *D. stramonium* seeds. There was no statistically significant difference between the groups [$F (1, 48) = 0.49$, $p = 0.487$] or between the groups and days [$F (5, 48) = 0.62$, $p = 0.685$]. During the first and fourth days of the training, the two groups virtually had equal escape latency time with no statistically significant value ($p = 0.713$). In the first and third training days, the treated groups showed lesser escape latency times when compared to their respective controls on the same days and still not significant statistically ($p > 0.05$). Similar observations were made in the second and fifth days of the escape latencies ($p > 0.05$) although the control group had a shorter time. In the third and sixth day (probe) days, the treated groups spent lesser escape latencies which were not significant ($p = 0.648$) in comparison to their respective controls.

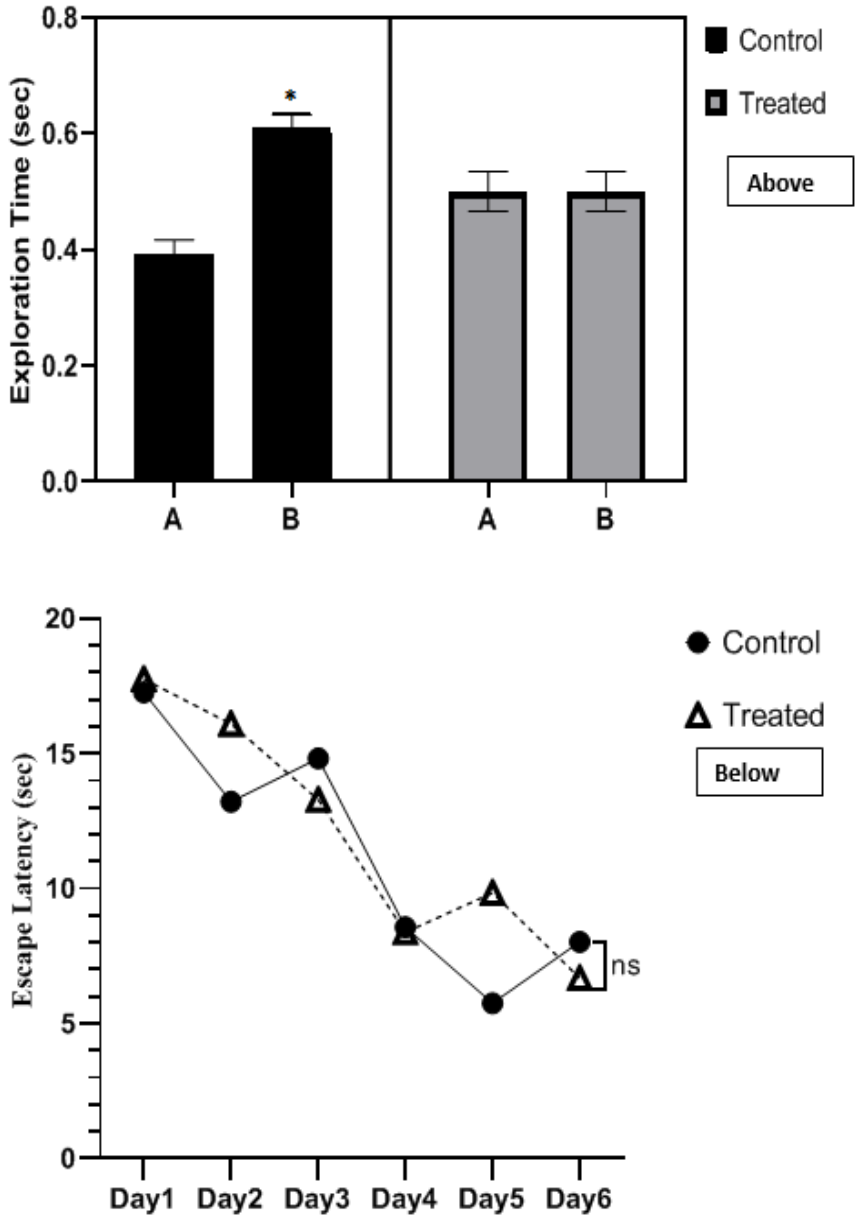

Figure 1. Novel object recogtion test (above) Morris water maze test (below) in Wistar rats following treatmentwith 800 mg/kgbwt hyoscyamine fraction of *D. stramonium* seeds at adolescence. No statistically significant (ns)(p = 0.648).

Plates IIA & B shows photomicrographs of CA1 regions in the hippocampus of adult Wistar rats at 12th week after being treated orally with equivalent body weight of normal saline at the and 800 mg/kgbwt hyoscyamine fraction of *D.stramonium* at adolescence from the PND 28 – 49 as control and treated groups respectively. The region in the control group showed normal histology of stratum oriens, (SO), stratum radiatum (SR), stratum lacunosum moleculare (SLM) and relatively normal pyramidal cell layer (PCL) density when compared to the treated group where, significant cytoplasmic vacuolations (black asterisks) with scattered, partly hyperchromic (black arrows) and apoptotic (black arrowhead) cells was observed in the region.

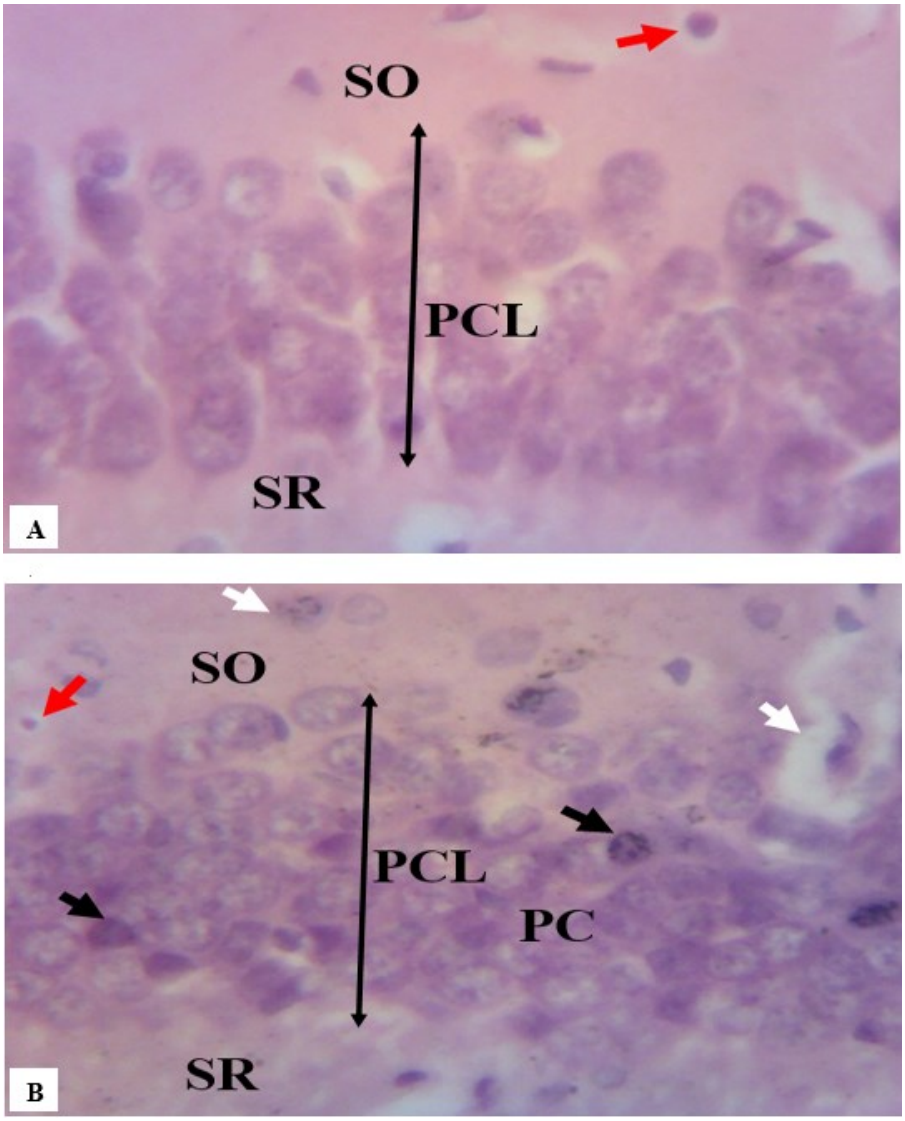

Plate II: A photomicrograph of CornuAmmonis (CA1) region of the hippocampus following administration of 800 mg/kgbwt hyoscyamine fraction of *D. stramonium* seeds at adolescence.

Discussion

Reports by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2017) have indicated an increased abuse of new psychoactive substances (NPS) by adolescents. Exposure to NPS during adolescence in man may have an impact on brain development and may lead to neuropsychiatric disorders in adulthood of man. Many drugs have been used for recreation purposes among adolescent and this includes cocaine (Estroff *et al*., 1989), ethanol (Spear, 2000). *Datura stramonium* is known for its psychoactive and anticholinergic properties (Das *et al*., 2012; Ujváry, 2014). The World Drug Report 2013 states a high lifetime prevalence rate of its use (2.6%) among adolescents (UN, 2013). Although, the escape latencies of the Morris water maze vary between groups but did not show significant difference between the groups on the test day. Both hippocampal and cortical lesions influence novel object recognition test paradigms (Clark *et al*., 2000; Buckmaster *et al*., 2004). The present study was similar to that of Olawepo *et al*. (2017), where a deficit in cognitive function was reported in adolescent rats following a single dose of atropine administration intra-peritoneally as compared with the control where, atropine was attributed to have affinity for rapidly dividing

cells (Shiu *et al.,* 2007; Chia-hua *et al.,* 2007, 2008; Li *et al.,* 2009; Lingmei *et al.,* 2010; Xia et. al., 2011; Xia *et al.,* 2012). The significant decrease in the memory observed was probably that generally, tropane alkaloids are believed to alter neurogenesis (Joels *et al.,* 2004; Joosen, 2009), by either involved in the destruction of the neural precursor cells thus limiting their survival rate and interfering with the cholinergic transmission, causes impairment in learning and short-term memory in rodents and humans (Iversen, 1997). The deficit in cognitive memory seen in the adulthood indicates that altered brain function at adolescent has a profound effect on the function of adult brain. The findings of this study were strengthened by the earlier reports of Nestle and Malenka (2004), Giedd (2004),Winters (2008), and Olawepo *et al.* (2017), that shows that drug abuse in adolescent enhances progression of neurodegenerative disorders at adulthood. Surprisingly our finding shows that the spatial memory was not affected in the treated animal when compared to the control. In a related finding, Olawepo *et al.* (2017) reported that a single dose of atropine administration intra-peritoneally at adolescent for three weeks, had no significant effects on Y-maze spatial learning ability test at the adulthood of the Wistar rats. This probably is an indication that spatial memory processing is different from temporal working memory, as it is often reported in the case of victims of atropine poisoning that are shown to still have the ability to remember places where they have been, but have anterograde amnesia following the poisoning (Holzman, 1998).

The hippocampus is important for memory based on careful studies in human patients with memory loss as a result of brain damage. The degree and type of memory deficits were correlated with the site of the brain lesion, as determined histologically, after autopsy. Such lesions involve the hippocampal formation as the most constant finding (Brodal, 2004). In the present study, the histology of the CA1 region of hippocampus in the treated group showed significant cytoplasmic vacuolations with scattered, partly hyperchromic and apoptotic cells was observed in the region. This was in tune with the similar results reported by Ekanem *et al*. (2016) where, ethanol extract of *D. stramonium* seeds found to cause atrophy of the axons and fibres, vacuolation, cell necrosis and cell losses in the pyramidal cells of the hippocampus. The study was also in consistent with the study carried out by Bihaqi *et al*. (2012) in which the histology of the cerebral cortex examined under light microscope showed increased neuronal loss, ghost cells, hemorrhage and vacuolated cytoplasm on treated rats that received daily intraperitoneal administration of scopolamine. The histological lesions observed could be attributed to the fact that various mediators can contribute to excitotoxin action, including production of reactive oxygen intermediates, nitric oxide, p53 and cytokines which could lead to a series of cascading events leading to cell losses in the hippocampus(Coyle & Puttfarcken, 1993; Franklin *et al*., 1994; Ankarcrona *et al*., 1995; Morrison *et al*., 1996), since all tropane alkaloids of *D. stramonium* have a long duration of effect, cross the blood brain barrier and have central anti-cholinergic effects (Bania *et al*., 2004) which induce strong hypnosis and can also induce neurodegeneration (Hughes &Clark, 1939).

Conclusion

The study concluded that exposure hyoscyamine fraction of D. stramonium at adolescence induces hippocampal cognitive deficit and neural cell death which may predisposes progression of neurodegenerative diseases at adulthood

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References

Adriani, W. and Laviola, G., 2004. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology*, 15(5), pp.341-352.

Adriani, W., Granstrem, O., Macri, S., Izykenova, G., Dambinova, S. and Laviola, G., 2004. P1 Behavioral and Neurochemical Vulnerability During Adolescence in Mice: Studies with Nicotine. *Behavioural Pharmacology*, 15(5), p.A8.

Anagnostaras, S., Maren, S. and Fanselow, M., 1999. Temporally Graded Retrograde Amnesia of Contextual Fear after Hippocampal Damage in Rats: Within-Subjects Examination. *The Journal of Neuroscience*, 19(3), pp.1106-1114.

Ankarcrona, M., Dypbukt, J., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. and Nicotera, P., 1995. Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. *Neuron*, 15(4), pp.961-973.

Bania, T., Chu, J., Bailes, D. and O'Neill, M., 2004. Jimson Weed Extract as a Protective Agent in Severe Organophosphate Toxicity. *Academic Emergency Medicine*, 11(4), pp.335-338.

Bihaqi, S., Singh, A. and Tiwari, M., 2012. Supplementation of Convolvulus pluricaulis attenuates scopolamine-induced increased tau and Amyloid precursor protein (AβPP) expression in rat brain. *Indian Journal of Pharmacology*, 44(5), p.593.

Blaney, C., Gunn, R., Stover, K. and Brown, R., 2013. Maternal genotype influences behavioral development of 3×Tg-AD mouse pups. *Behavioural Brain Research*, 252, pp.40-48.

Brodal, P., 2004. *The Central Nervous System: Structure And Function*. 1st ed. USA, New York: Oxford University Press, p.515.

Buckmaster, C., 2004. Entorhinal Cortex Lesions Disrupt the Relational Organization of Memory in Monkeys. *Journal of Neuroscience*, 24(44), pp.9811-9825.

Christie, D. and Viner, R., 2005. Adolescent development. *BMJ*, 330(7486), pp.301-304.

Clark, R., Zola, S. and Squire, L., 2000. Impaired Recognition Memory in Rats after Damage to the Hippocampus. *The Journal of Neuroscience*, 20(23), pp.8853-8860.

Coyle, J. and Puttfarcken, P., 1993. Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, 262(5134), pp.689-695.

Dahl, R., 2004. Adolescent Brain Development: A Period of Vulnerabilities and Opportunities. Keynote Address. *Annals of the New York Academy of Sciences*, 1021(1), pp.1-22.

Das, S., Kumar, P. and Basu, S., 2012. Phytoconstituents And Therapeutic Potentials of Datura Stramonium Linn. *Journal of Drug Delivery and Therapeutics*, 2(3).

Ding, X., Zhu, F., Li, M. and Gao, S., 2012. Induction of apoptosis in human hepatoma SMMC-7721 cells by solamargine from Solanum nigrum L. *Journal of Ethnopharmacology*, 139(2), pp.599-604.

Djilani, A., Legseir, B., Soulimani, R., Dicko, A. and Younos, C., 2006. New extraction technique for alkaloids. *Journal of the Brazilian Chemical Society*, 17(3), pp.518-520.

Ekanem, P., Ekanem, R. and Gaim, K., 2016. Histological Patterns of Neurodegeneration of Frontal Cortex Neurons in <i>Daturastramonium</i> Treated Wistar Rats. *Journal of Behavioral and Brain Science*, 06(02), pp.85-92.

EMCDDA.europa.eu. 2020. *2017 European Drug Report | Www.Emcdda.Europa.Eu*. [online] Available at: <https://www.emcdda.europa.eu/edr2017_en> [Accessed 21 August 2020].

Epstein, F., Lipton, S. and Rosenberg, P., 1994. Excitatory Amino Acids as a Final Common Pathway for Neurologic Disorders. *New England Journal of Medicine*, 330(9), pp.613-622.

Estroff, T., Schwartz, R. and Hoffmann, N., 1989. Adolescent cocaine abuse. Addictive potential, behavioral and psychiatric effects. *Clinical Pediatrics*, 28(12), pp.550-555.

Frankland, P. and Bontempi, B., 2005. The organization of recent and remote memories. *Nature Reviews Neuroscience*, 6(2), pp.119-130.

Giedd, J., 2004. Structural Magnetic Resonance Imaging of the Adolescent Brain. *Annals of the New York Academy of Sciences*, 1021(1), pp.77-85.

Guderian, S., Dzieciol, A., Gadian, D., Jentschke, S., Doeller, C., Burgess, N., Mishkin, M. and Vargha-Khadem, F., 2015. Hippocampal Volume Reduction in Humans Predicts Impaired Allocentric Spatial Memory in Virtual-Reality Navigation. *Journal of Neuroscience*, 35(42), pp.14123-14131.

Hefner, K. and Holmes, A., 2007. Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. *Behavioural Brain Research*, 176(2), pp.210-215.

Henry, T., 1949. *The Tropane Alkaloids. In The Plant Alkaloids*. London: JA Churchill Ltd, pp.69–118.

Holzman, R., 1998. The Legacy of Atropos, the Fate Who Cut the Thread of Life. *Anesthesiology*, 89(1), pp.241-249.

Hughes, J. and Clark, J., 1939. Stramonium Poisoning. *Journal of the American Medical Association*, 112(24), p.2500.

Ishola, A. and Adeniyi, P., 2013. Retarded hippocampal development following prenatal exposure to ethanolic leaves extract of Datura metel in wistar rats. *Nigerian Medical Journal*, 54(6), p.411.

Iversen, S., 1997. Behavioural evaluation of cholinergic drugs. *Life Sciences*, 60(13-14), pp.1145-1152.

Jablonski, S., Schreiber, W., Westbrook, S., Brennan, L. and Stanton, M., 2013. Determinants of novel object and location recognition during development. *Behavioural Brain Research*, 256, pp.140-150.

Joëls, M., Karst, H., Alfarez, D., Heine, V., Qin, Y., Riel, E., Verkuyl, M., Lucassen, P. and Krugers, H., 2004. Effects of Chronic Stress on Structure and Cell Function in Rat Hippocampus and Hypothalamus. *Stress*, 7(4), pp.221-231.

Johnson, C. and Wilbrecht, L., 2011. Juvenile mice show greater flexibility in multiple choice reversal learning than adults. *Developmental Cognitive Neuroscience*, 1(4), pp.540-551.

Joosen, M., Jousma, E., van den Boom, T., Kuijpers, W., Smit, A., Lucassen, P. and van Helden, H., 2009. Long-term cognitive deficits accompanied by reduced neurogenesis after soman poisoning. *NeuroToxicology*, 30(1), pp.72-80.

Kamada, H., Okamura, N., Satake, M., Harada, H. and Shimomura, K., 1986. Alkaloid production by hairy root cultures in Atropa belladonna. Plant Cell Reports, 5(4), pp.239-242.

Laviola, G., Macrì, S., Morley-Fletcher, S. and Adriani, W., 2003. Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience & Biobehavioral Reviews*, 27(1-2), pp.19-31.

Li, G., Bien-Ly, N., Andrews-Zwilling, Y., Xu, Q., Bernardo, A., Ring, K., Halabisky, B., Deng, C., Mahley, R. and Huang, Y., 2009. GABAergic Interneuron Dysfunction Impairs Hippocampal Neurogenesis in Adult Apolipoprotein E4 Knockin Mice. *Cell Stem Cell*, 5(6), pp.634-645.

Liang, C., Shiu, L., Chang, L., Sheu, H. and Kuo, K., 2007. Solamargine upregulation of Fas, downregulation of HER2, and enhancement of cytotoxicity using epirubicin in NSCLC cells. *Molecular Nutrition & Food Research*, 51(8), pp.999-1005.

Liang, C., Shiu, L., Chang, L., Sheu, H., Tsai, E. and Kuo, K., 2008. Solamargine Enhances HER2 Expression and Increases the Susceptibility of Human Lung Cancer H661 and H69 Cells to Trastuzumab and Epirubicin. *Chemical Research in Toxicology*, 21(2), pp.393-399.

Lillie R. 1965. Histopathologic Technic and Practical Histochemistry. 3rd ed. New York: Blakiston Division, Mcgraw-Hill.

Lorke, D., 1983. A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4), pp.275-287.

Maguire, E., Burgess, N. and O'Keefe, J., 1999. Human spatial navigation: cognitive maps, sexual dimorphism, and neural substrates. *Current Opinion in Neurobiology*, 9(2), pp.171-177.

Maguire, E., Gadian, D., Johnsrude, I., Good, C., Ashburner, J., Frackowiak, R. and Frith, C., 2000. Navigation-related structural change in the hippocampi of taxi drivers. *Proceedings of the National Academy of Sciences*, 97(8), pp.4398-4403.

Mills, K., Lalonde, F., Clasen, L., Giedd, J. and Blakemore, S., 2014. Developmental changes in the structure of the social brain in late childhood and adolescence. *Social Cognitive and Affective Neuroscience*, 9(1), pp.123-131.

Molenhuis, R., de Visser, L., Bruining, H. and Kas, M., 2014. Enhancing the value of psychiatric mouse models; differential expression of developmental behavioral and cognitive profiles in four inbred strains of mice. *European Neuropsychopharmacology*, 24(6), pp.945-954.

Moore, E., Linsenbardt, D., Melón, L. and Boehm, S., 2011. Ontogenetic differences in adolescent and adult C57BL/6J and DBA/2J mice: Anxiety-like, locomotor, and consummatory behaviors. *Developmental Psychobiology*, 53(2), pp.141-156.

Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, 11(1), pp.47-60.

Morrison, R., Wenzel, H., Kinoshita, Y., Robbins, C., Donehower, L. and Schwartzkroin, P., 1996. Loss of the p53 tumor suppressor gene protects neurons from kainate- induced cell death. *The Journal of Neuroscience*, 16(4), pp.1337-1345.

Moscovitch, M., Nadel, L., Winocur, G., Gilboa, A. and Rosenbaum, R., 2006. The cognitive neuroscience of remote episodic, semantic and spatial memory. *Current Opinion in Neurobiology*, 16(2), pp.179-190.

NDLEA. Journals.oouagoiwoye.edu.ng. 1997. *Drug Data Collection And Research, Lagos: Drug Demand Reduction Unit, National Drug Law Enforcement Agency*. [online] Available at: <https://journals.oouagoiwoye.edu.ng/v2/index.php/JSBS/article/view/9> [Accessed 21 August 2020].

Nestler, E. and Malenka, R., 2004. The Addicted Brain. *Scientific American*, 290(3), pp.78-85.

Olawepo, A., Ishola, A., Ajao, M., Olayemi, O. and Olayaki, L., 2017. Atropine exposure in adolescence predispose to adult memory loss in Wistar rats. *International Journal of Biological and Chemical Sciences*, 11(5), p.1937.

Paus, T., 2005. Mapping brain maturation and cognitive development during adolescence. *Trends in Cognitive Sciences*, 9(2), pp.60-68.

Qing, H., He, G., Ly, P., Fox, C., Staufenbiel, M., Cai, F., Zhang, Z., Wei, S., Sun, X., Chen, C., Zhou, W., Wang, K. and Song, W., 2008. Valproic acid inhibits Aβ production, neuritic plaque formation, and behavioral deficits in Alzheimer's disease mouse models. *Journal of Experimental Medicine*, 205(12), pp.2781-2789.

Romeo, R., Richardson, H. and Sisk, C., 2002. Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. *Neuroscience & Biobehavioral Reviews*, 26(3), pp.381-391.

Shiu, L., Chang, L., Liang, C., Huang, Y., Sheu, H. and Kuo, K., 2007. Solamargine induces apoptosis and sensitizes breast cancer cells to cisplatin. *Food and Chemical Toxicology*, 45(11), pp.2155-2164.

Sisk, C. and Zehr, J., 2005. Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology*, 26(3-4), pp.163-174.

Spear, L., 2000. The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*, 24(4), pp.417-463.

Spear, L., 2000. The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*, 24(4), pp.417-463.

Squire, L., Stark, C. and Clark, R., 2004. The Medial Temporal Lobe. Annual Review of Neuroscience, 27(1), pp.279-306.

Sun, L., Zhao, Y., Li, X., Yuan, H., Cheng, A. and Lou, H., 2010. A lysosomal–mitochondrial death pathway is induced by solamargine in human K562 leukemia cells. *Toxicology in Vitro*, 24(6), pp.1504-1511.

Tirelli, E., Laviola, G. and Adriani, W., 2003. Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neuroscience & Biobehavioral Reviews*, 27(1-2), pp.163-178.

Tirelli, E., Laviola, G. and Adriani, W., 2003. Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neuroscience & Biobehavioral Reviews*, 27(1-2), pp.163-178.

Ujváry, I., 2014. Psychoactive natural products: Overview of recent developments. *Annual 1st Super Sanita*, 50, pp.12-27.

United Nations: Office on Drugs and Crime. 2010. *WDR-2010*. [online] Available at: <https://www.unodc.org/unodc/en/data-and-analysis/WDR-2010.html> [Accessed 21 August 2020].

UNODC.org. 2020. *World Drug Report 2013*. [online] Available at: <https://www.unodc.org/lpo-brazil/en/drogas/relatorio-mundial-sobre-drogas.html> [Accessed 22 August 2020].

Wiltgen, B., Sanders, M., Anagnostaras, S., Sage, J. and Fanse-low, M., 2006. Context Fear Learning in the Absence of the Hippocampus. *Journal of Neuroscience*, 26(20), pp.5484-5491.

Winters, K., 2008. *Adolescent Brain Development And Drug Abuse*. Mentor Foundation Online Book.

Xia, L., Ying, Z., Mei, J., Shan-Shan, L., Min, C. and Hong-Xiang, L., 2011. Induction of actin disruption and downregulation of Pglycoprotein expression by solamargine in multidrug-resistant K562/AO2 cells. *Chinese Medical Journal (Engl.)*, 124(13), pp.2038-2044.

APPENDIX – I

AHMADU BELLO UNIVERSITY, ZARIA
DIRECTORATE OF ACADEMIC PLANNING & MONITORING
Vice Chancellor Prof. Ibrahim Garba, B.Sc. (Hons) Geology, M.Sc. (Mineral Exploration) A.R.C., Ph.D Geology (London), F.I.G.C., FNIGS
Director: Prof. M.F. Ishiyaku, B.Sc. (Hons) Botany (ABU), M.Sc. Plant Breeding (Oxford), Ph.D Agriculture (University of Reading, U.K.), MASN, MBSN
Tel. Zaria + 234-069-551912 Fax. 234 (069) 550822 Telegram: UNIBELLO Zaria e-mail dapm@abu.edu.ng

Appl No.: ABUCAUC/2018/Human Anatomy/042
Approval No: ABUCAUC/2018/042

3rd October, 2018

Dr. S. A. Musa
Department of Human Anatomy,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Ahmadu Bello University,
Zaria.

Sir,

APPROVAL OF RESEARCH STUDY 'EVALUATION OF INGESTION OF HYOSCYAMINE FRACTIONATE OF DATURA STRAMONIUM SEEDS ON CEREBRUM DEVELOPMENT IN WISTAR RATS *(RATTUS NORVEGICUS)*

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Human Anatomy. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

U.D. Abdullahi
For: Chairman, ABUCAUC.

Cc. Director, DAPM
Director, IC &ICT
Provost, College of Health Sciences
Dean, Faculty of Basic Medical Sciences,
HOD, Human Anatomy,
Prof. Aliyu Mohammed