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EVALUATION OF THE EFFECTS OF LACTATIONAL EXPOSURE TO HYOSCYAMINE FRACTION OF *DATURA STRAMONIUM* L. SEEDS ON LEARNING AND MEMORY IN WISTAR RATS (*RATTUS NORVEGICUS*)

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Ethics committee approval: The ethics committee approval has been obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2018/042).

Abstract

The study was designed to determine whether lactational exposure to hyoscyamine fraction of *Datura stramonium* L. (*D. stramonium*) seeds affect the cognitive, spatial learning and memory functions of the hippocampus in Wistar rats at adulthood. Fresh seeds of *D. stramonium* L. were procured, macerated and fractionated using high-performance liquid chromatography (HPLC). Eight (8) Wistar rats weighed 150-250 grams of equal gender were used for the study. The rats were mated and divided into control and treatment groups. Equivalent body weight of normal saline and 400 mg/kgbw of hyoscyamine fraction were orally administered to the breastfeeding rats respectively on lactational days (LD) 1-21. At adulthood, the rats were subjected to neurobehavioural tests using novel objects recognition (NORT) and Morris water maze (MWM) respectively. The data obtained were expressed as mean \pm SEM, independent two samples t-test and General Linear Model (GLM) repeated-measures ANOVA with Fisher's multiple comparisons post-hoc tests were used to show the mean differences using Minitab 17 (LLC., U.K.) statistical package software. $P < 0.05$ was considered statistically significant. A significant increase in the meantime of exploration between the groups ($p = 0.049$) was observed during the NORT. No statistically significant increase ($p = 0.626$) in the meantime to locate the escape platform between the groups during the MWM test. The CA1 region of the treated group showed mild nuclear hyperchromasia, cytoplasmic vacuolations and pyknosis. In conclusion, exposure to hyoscyamine fraction of *D. stramonium* L. seeds at lactation caused histologic changes in the CA1 region, loss in short-term memory but not spatial learning and memory functions of the hippocampus of Wistar rats at adulthood.

Keywords: *D. stramonium*, hippocampus, histology, hyoscyamine, lactation

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1. Introduction

Breastfeeding has an advantage for both infants and mothers. It provides the nutritional requirement of developing of infants (WHO, 2013). It gives optimal nutrition and improved mental performance and neurological development (Onyiaapat *et al.*, 2011). It also enhances immunity (Okoronkwo *et al.*, 2014; Bodeker *et al.*, 2002) of the developing infants. It decreases the chances of an unexpected death of infants, allergic diseases, and development of Type-1 and Type-2 diabetes mellitus (Zhang *et al.*, 2012; Achigbu & Achigbu, 2014; Okoronkwo *et al.*, 2014; Alwhaibi and Sambamoorthi, 2016) when compared to infant formula. Breastfeeding reduces postpartum depression, bleeding, and improves weight control (Kaadaaga *et al.*, 2014). Transfer of medical substances by the breastfeeding women (Zhang *et al.*, 2012; Kaadaaga *et al.*, 2014) to their babies is a matter of concern. In humans, the medicines that circulate in the maternal bloodstream can be transferred to their babies through lactation, hence, exposing the infants to such medicines which may potentially be harmful (Zhang *et al.*, 2012; Kaadaaga *et al.*, 2014). Some of the conventional medicines indicated to have compromised milk production include cabergoline (Tsai *et al.*, 2012), bromocriptine (Chien *et al.*, 2006), ergotamine (Ebrahim *et al.*, 2012), pseudoephedrine (Gatti, 2008), and oestrogens (Ebrahim *et al.*, 2012; Jackson, 2010). Nowadays, the patronage of traditional herbs is on the rise globally. Many developed countries across the globe including the United States (Sibeko *et al.*, 2005; Kimani-Murage *et al.*, 2015), Canada (Ranasinghe *et al.*, 2015), the United Kingdom (Diaz *et al.*, 2013; James *et al.*, 2018), United Arab Emirates (James *et al.*, 2018) and Australia (Nordeng *et al.*, 2013; Macfoy, 2013; Mothupi, 2014; Pieterse and Lodge, 2015; James and Bah, 2016 and 2018; Witter *et al.*, 2016), have reported significant patronage of traditional herbs amongst the general population. The trend is similar in Africa such that either modern health care and medicine is often available only to a limited number of people, the facilities are too expensive or too few to cater the needs of too many people (Ahmed *et al.*, 2018).

Datura stramonium, a family member of Solanaceae, an annual plant that possesses phytoconstituents, with alkaloids possessing strong anticholinergic properties (Berger & Ashkenazi, 2003; Nuhu & Ghani, 2002; Oberndorfer *et al.*, 2002; Ruhwald, 2005). They competitively antagonize acetylcholine at peripheral and central muscarinic receptors for the common binding site (Friedman and Levin, 1989; Ruhwald, 2005). Its leaves are used to bust-up sagged breast among Pakistan women, while seeds are taken in a cup of green tea to relieve headache (Hussain *et al.*, 2006).

In Nigeria, the juice of *Datura* leaves mix with warm milk is used to expel intestinal worms (Egharevba and Ikhatua, 2008), while the seeds in palm oils are used for external treatment of insect bites and stings (Rajbhandari, 2001). Both seeds and leaves of *Datura* are consumed by the youths as a part of the local beverage (Zobo) or in porridge for recreation at ceremonies and public stroke-beating (Shadi/Sharo) in the suitors of Fulani tribe. This marks a sign of courage and responsibility to the suitress and its family. Pieces of literature have reported that

D. stramonium decreases the production of breast milk (Barrager *et al.*, 2002), however, little was reported as to whether it is safe or harmful to the nursing infant (Mills, 2006) if consumed at lactation.

The current study aimed to find out whether maternal ingestion of hyoscyamine fraction of *D. stramonium* L. seeds at lactation affects the cognitive function of the hippocampus in Wistar rats at adulthood. The study may provide awareness on the risk associated with the ingestion of psychoactive ethnomedicinal plants such as *D. stramonium* L. seeds during lactation on the memory functions of the hippocampus.

2. Materials and Methods

The Ethics committee approval: The study was approved by the ethics committee of Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2018/042) which is in line with the Declaration of Helsinki.

2.1. Plant materials

Fresh *D. stramonium* seeds were 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 powdered sample was collected into a sterile cellophane bag and kept in a cool dry place for extraction.

2.2. Ethanol extraction of crude *D. stramonium* seeds

Extraction was carried out using cold maceration according to Djilani *et al.*, 2006. The 200g of pulverized seeds were soaked in 1, 500 ml of 70% (v/v) ethanol at room temperature and allowed to macerate for 72 hrs. The extract was filtered and the solvent was evaporated in a water-bath at 40 °C. The residue, dissolved in 250 ml H₂O and acidified with few drops H₂SO₄ to pH 3-4, extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral material, and basified with the aqueous solution of NH₄OH (0.25M) at pH 9-10. The extract was washed with distilled water to neutral pH, dried with Na₂SO₄ and concentrated to dryness under reduced pressure to obtain crude alkaloids.

2.3. Fractionation of hyoscyamine

The fractionation was carried out according to Salamah and Ningsih, 2017. Five grams of the viscous extract was dissolved in 10 ml of water. The solution was then poured into a separating funnel, added with 10 ml of chloroform, and shaken to solve with two phases, namely water and chloroform. These two phases were separated and collected. This was repeated until the chloroform phase had the same colour as the chloroform solvent. The chloroform was then evaporated and recrystallized to obtain the hyoscyamine fraction. The alkaloid was analyzed with UV-Vis spectrophotometric method. The extraction and fractionation were carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

2.4. Quantification of hyoscyamine fraction

HPLC Condition

A reversed-phase Techsphere 50DS C18 HPLC column (25 cm × 4.6 mm i.d.) particle size 5 µm, Supelco, Bellefonte, PA, USA) with oven temperature, 40°C in conjunction with UV adsorption detector operating at 270 nm was employed. The mobile phase was a mixture of 20% acetonitrile, and 45 % methanol, 35 % water (H₂O) and 0.1 mol/L phosphoric acids which adjusted the pH to 7.0 and flow rate of 1 ml/min. A calibration curve for l-hyoscyamine was plotted to determine the amount of the hyoscyamine in the sample fraction. All analyses were carried out at the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

2.5. Experimental animals

Eight (8) healthy Wistar rats comprised of equal numbers of adult males and virgin females were procured from the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria. The animals were transported to the Animal House of the Pharmacology Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) Zaria, Kaduna State, Nigeria. The males were separated from the females, housed and allowed to acclimatize for two weeks at ambient temperature, with alternate day and night cycles at the natural condition. Rat chow (Vital feeds®) and tap water were made available to the animal's *ad libitum*. The median lethal dose (LD₅₀) of hyoscyamine fraction was determined using Lorke's (1983) method. Neurotoxicity symptoms were observed and the animals were allowed for twenty-four hours to be observed for mortality.

2.6. Synchronization and phase determination of the oestrus cycle and mating

The female rats were injected with an equivalent bodyweight of Zoladex® (3.6 mg AstraZeneca) intraperitoneally to synchronize their oestrus cycle. Twenty-four after the synchronization, vaginal smears were collected by vaginal lavage (Michelle, 2015) using a 1 ml plastic pipette filled with 10 µL of normal saline (NaCl 0.9%). The tip of the pipette was gently but superficially inserted into the rat vagina (Marcondes *et al.*, 2002). The vaginal fluid was carefully aspirated and placed on a cleaned glass slide. A different glass slide was used for each rat, and unstained material was viewed under a light microscope, without the use of the condenser lens, at 10 x objective lenses. The proportion of the round-nucleated cells, cornified cells, the leukocytes among them was used to determine phases of the oestrous cycle (Marcondes *et al.*, 2002).

2.7. Experimental design

The animals were randomly selected and divided into two (2) groups; control and treatment. Each group contained a total of eight (8) rats in the ratio of 1:1 adult male to virgin female. Animals in each group were allowed to mate freely and evidence of mating was established by the presence of sperms tails in the vaginal smears collected and viewed under a light microscope after 24 hrs (figure 1b). Abdominal palpation was carried out to avoid error

due to pseudopregnancy. The pregnant dams detected, were isolated and transferred to maternity cages. Animal from the same group was kept closely but separately together in different cages. A total of forty pups (40) were obtained at after parturition. The control and treatment groups received an equivalent bodyweight of a single dose of normal saline and 400 mg/kgbw of hyoscyamine fraction of *D. stramonium* seeds respectively orally daily for three weeks, from the lactational day (LD) 1-21. After treatment, the animals were nurtured to adulthood (PND 75).

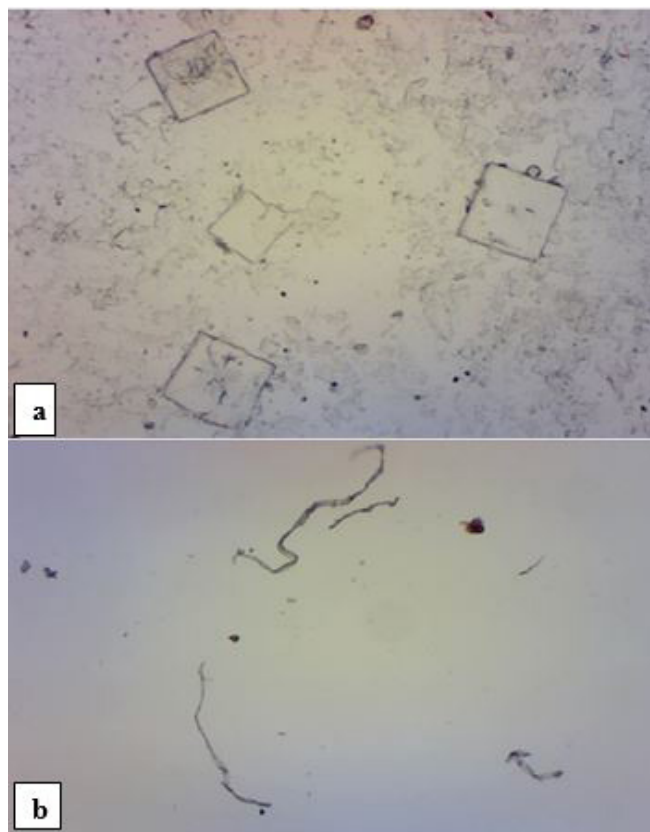


Figure 1 a&b. Proestrous phase and sperm tails in vaginal smears of Wistar rats showing nucleated leukocytes and sperm tails 24 hrs after mating (x 10 magnification).

2.8. Novel object recognition test

This was conducted at adulthood (PND 60 -75). The aim was to test short-term memory according to Gaskin *et al.*, 2010. 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 the habituation session, a digital and a video camera (Model DCR – PJ5E, SONY®) was used for proper video coverage of the rats' activity 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 the 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 (A1 and A2) and two unidentical objects (A and B) objects for were used 15 minutes respectively. The object bias score was calculated according to Ennaceur and Delacour (1988).

2.9. Morris water maze

This was carried out also at adulthood (PND 60 – 75) for six consecutive days using Morris (1984) protocol to test spatial learning and memory. 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 to about the two-third depth of the tank at 22 – 25°C, deep enough to expose 2.54 cm (1 inch) of the platform above the water surface. A digital video device (Model DCR – PJ5E, SONY®) was suspended directly over the pool to capture the entire setup. The rats were trained for 5 days with methylene-blue stained water that submerged the platform 1 inch beneath except for the pretraining day1, where the platform was 1 inch above the clean and clear water. A latency period of 60 sec was allowed for each rat 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. On day 6, the test day, the setup was maintained as the previous days except that 30 seconds per trial with no escape platform was observed. The time taken for each rat to identify the usual position of the platform was recorded and all videos recorded for the trials were analyzed for the escape latency.

2.10. 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 (graded alcohol) and cleared (in xylene) using an automatic processing machine (Shandon Southern Duplex Processor). The tissues were embedded in paraffin wax and blocked in the coronal plane. Serial sections of the blocks were taken at 8 µm with a microtome (Leitz Wetzlar), mounted on glass slides and allowed to dry overnight. The staining technique employed was hematoxylin and eosin in paraffin sections (Lillie and Fullmer, 1976). Sections were viewed 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). The sections of the hippocampus were observed in the treated rats and compared to the controls.

2.11. Statistical Analyses

The data obtained were expressed as mean ± SEM. Independent samples – t-test and pairwise General Linear Model (GLM) repeated measures 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. $P < 0.05$ was considered statistically significant. All figures and charts were constructed using GraphPad Prism 8.

3. Results

No mortality was observed in the first phase when the animals received 10, 100 and 1,000 mg/kgbw. However, toxicity symptoms characterized by restlessness (hyperactivity), laboured breathing, piloerection, abdominal cramps, stooling (diarrhoea), and urination, were observed especially at the 1,000 mg/kgbw. The symptoms later disappeared and the animals became calm, weak and quiet. During the second phase, the symptoms persisted with high intensity in all the groups treated with 1,600, 2,900 and 5,000mg/kgbw. Neurotoxicity symptoms were observed but, no mortality was recorded even at the highest dose. The *D. stramonium* fraction was therefore considered safe in Wistar rats and 5000 mg/kgbw was taken as the LD50.

Figure 2 shows percentage exploration time in the sampling phase of cognitive function test using novel objects recognition test (NORT) using two identical objects (A1 and A2) between the control, and adult Wistar rats treated with 400 mg/kgbw hyoscyamine treated groups. There was no statistically significant difference ($p > 0.05$) in the exploration time for objects exploration. Any rat that scored less than or above 20 or 80% respectively was excluded for the test-phase of the experiment.

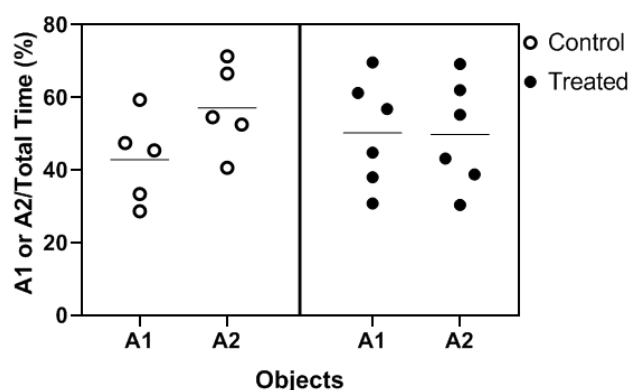


Figure 2. Sampling phase of Novel object recognition in Wistar rats exposed to an equivalent bodyweight of normal saline and 400 mg/kgbw hyoscyamine fraction of *D. stramonium* seeds at lactational day (LD)1-21.

The result of comparisons of the novel object recognition test in the test-phase between the control and treated groups was shown in figure 3. The time spent to explore the novel object (B) decrease significantly ($p = 0.049$) compared to the familiar (A). Although, there was an increase in the time taken to explore novel object (B) in the treated group but was not statistically significant ($p = 0.238$).

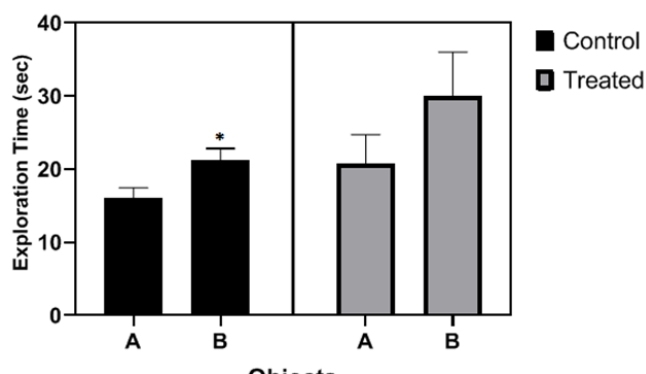


Figure 3. Test-phase in the novel object recognition test in Wistar rats exposed to an equivalent bodyweight of normal saline and 400 mg/kgbw hyoscyamine fraction of *D. stramonium* seeds at lactational day (LD)1-21. * $p = 0.049$.

Figure 4 comparison of discrimination index in novel object between the control and treated groups. No statistically significant difference ($p > 0.05$) in the discrimination ability between the groups.

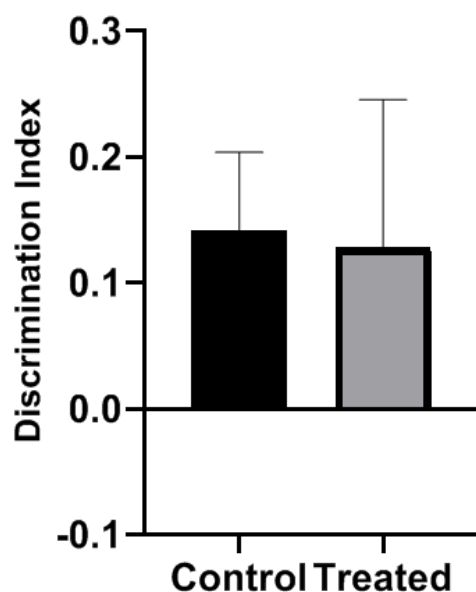


Figure 4. Discrimination index of novel object recognition test in Wistar rats exposed to an equivalent bodyweight of normal saline and 400 mg/kgbw hyoscyamine fraction of *D. stramonium* seeds at lactational day (LD)1-21.

In figure 5 independent two samples t-test of novelty preference between the groups. The time taken to explore the novel object between the groups was not statistically significant ($p = 0.411$). Thus, the control and the treated had an equal preference for the novel object.

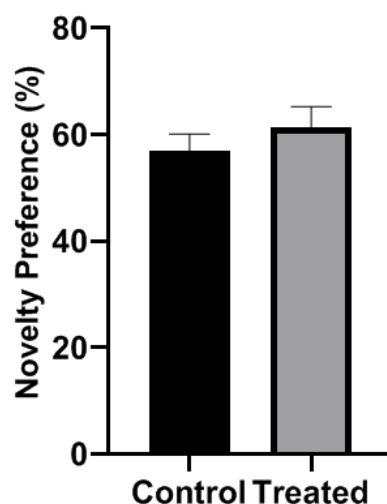


Figure 5. Novelty preference in Wistar rats exposed to an equivalent bodyweight of normal saline and 400 mg/kgbw hyoscyamine fraction of *D. stramonium* seeds at lactational day (LD)1-21

Figure 6 shows a comparison of the spatial learning and memory test. No significant differences in the time taken to locate the escape platform between groups [$F(1, 54) = 0.02$, $p = 0.875$] or between groups and days [$F(5, 54) = 0.64$, $p = 0.670$]. The post-hoc test further showed that during the first and third training days the treated groups showed lesser escape latencies when compared to the controls, there was however no statistically significant ($p > 0.05$) differences in the spatial learning was observed when compared to the controls. Similar observations were made in the control group on the second and fifth training days of the training ($p > 0.05$). On the fourth training day, the two groups located the escape platform at an equal time with no statistically significant ($p = 0.799$) difference in the escape latencies. On the sixth day (probe), the escape latency time was shorter in the treated compared to the control group but not significant statistically ($p = 0.626$).

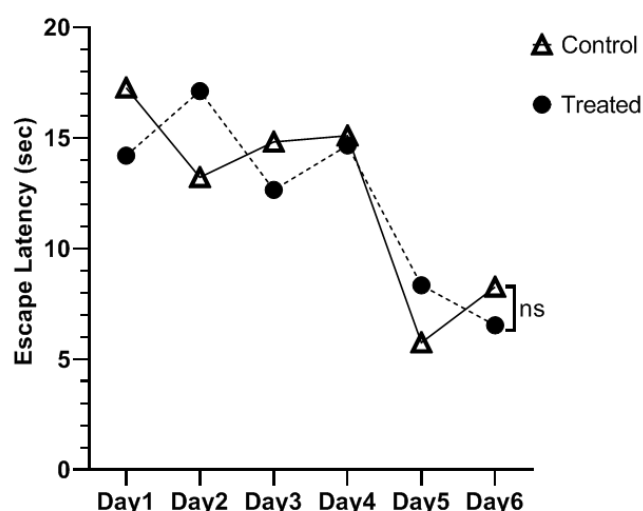


Figure 6. Morris water maze test in Wistar rats exposed to an equivalent bodyweight of normal saline and 400 mg/kgbw hyoscyamine fraction of *D. stramonium* seeds at lactational day (LD)1-21. $p = 0.626$.

Figure 7 a&b, H&E photomicrographs of CA1 regions in adult Wistar rats at 12th weeks after being treated with normal saline and hyoscyamine fraction of *D. stramonium* L. seeds from the lactational day (LD) 1 – 21 as control and treated groups respectively. The control group showed normal histology of the SO, SR and PCL with slight patches of oligodendrocytes as compared to the treated group were, slight patches of smaller but mild nuclear hyperchromasia nuclei (black arrows), mild cytoplasmic vacuolations (black asterisk) and mild pyknotic cells (white arrowhead).

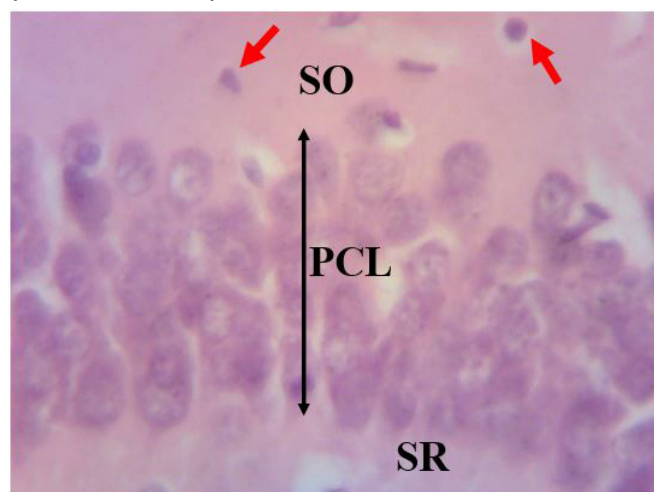


Figure 7a. Cornu Ammonis (CA1) region of the hippocampus in Wistar rats exposed to an equivalent bodyweight of normal saline at lactational day (LD)1-21. (H&E, x 400). PCL = Pyramidal Cell Layer, SO = stratum oriens, SR = stratum radiatum, Δ = pyknotic pyramidal cells, \blacktriangleleft = hyperchromic granular cells \rightarrow = oligodendrocytes.

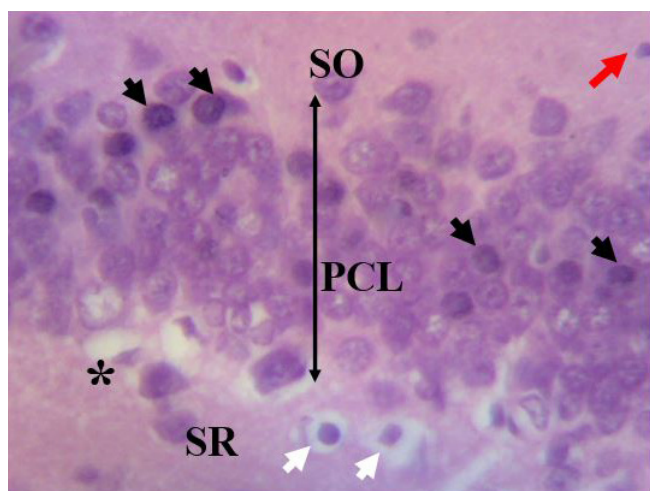


Figure 7b. Cornu Ammonis (CA1) region of hippocampus in Wistar rats exposed 400 mg/kgbw hyoscyamine fraction of *D. stramonium* seeds at lactational day (LD)1-21. (H&E, x 400). PCL = Pyramidal Cell Layer, SO = stratum oriens, SR = stratum radiatum, Δ = pyknotic pyramidal cells, \blacktriangleleft = hyperchromic granular cells \rightarrow = oligodendrocytes, * = cytoplasmic vacuolations.

4. Discussion

Ingestion of hyoscyamine fraction resulted in both

central and peripheral neurotoxicity symptoms in the rats fed with graded doses of the fraction. However, no mortality was recorded as a result of ingestion both during toxicity testing and experiment itself. In a related study by Babalola *et al.* (2015) reported that the median toxic dose of *D. stramonium* fed orally in dogs was at the safety margin as considered Centre for Disease Control (CDC) the United State of America, states. However, no published literature works available to make a comparison of the current study in Wistar rats. Considering the foregoing it could be assumed that oral ingestion of *D. stramonium* seeds might have a high safety margin in Wistar rats. The clinical symptoms observed might probably result from the anticholinergic properties of tropane alkaloids which competes and irreversibly inhibits acetylcholine on muscarinic receptors, thereby causing both central and peripheral nervous system manifestations (Hanna *et al.*, 1992). The central nervous system features include restlessness (hyperactivity), laboured breathing and delirium, while the peripheral symptoms observed include breathing, piloerection, abdominal cramps, stooling (diarrhoea), and urination. Similar observations were reported in patients involved in *D. stramonium* poisoning (Ramirez *et al.*, 1999).

Hyoscyamine accounts for 66% of the total tropane alkaloid content (El Bazaoui, 2011) with about 99% of the analyzed *Datura* seeds as (-)-hyoscyamine (Marín-Sáez *et al.*, 2016). The antimuscarinic activity of hyoscyamine is stereospecifically caused by the (-)-hyoscyamine enantiomer which was estimated to be more potent than the (+)-enantiomer (FAO/WHO, 2020). Acetylcholine (ACh) is one among the main neurotransmitters that cause changes in the brain memory function. It has numerous receptors that are found in various tissue involved in learning and memory (VanPatten & Al-Abed, 2016). Hyoscyamine competes for acetylcholine by binding to muscarinic receptors the nervous systems. The ability of tropane alkaloids to cause a change in neurogenesis have been reported with unclear mechanisms of action (Joels *et al.*, 2004; Joosen, 2009). The current study did not observe a statistically significant increase in the novel object recognition test (NORT) during the novel object discrimination or novelty preference between the groups except for the exploration time where the significant increase was observed in the control group, thus more curiosity towards the novel object. There was also, no statistically increase in the time taken by the treated rats to locate the escape platform during spatial learning and memory test of the Morris water maze. To the best of our knowledge, this probably is the first report that evaluates the effects of exposure to hyoscyamine fraction of *D. stramonium* seeds on Wistar rats treated at the lactation stage, for neurobehavioral impairments. The lack of cognitive deficit observed in the adulthood perhaps probably indicates that hippocampus exposure to hyoscyamine fraction of *D. stramonium* seeds does not affect the memory functions of the hippocampus at adulthood in Wistar rats. In a similar study, however, adolescent Wistar rats exposed to atropine were reported to suffer a deficit in hippocampus cognitive function at adulthood (Olawepo *et al.* (2017). This discrepancy might be attributed to the route of exposure and enantiomerization during the extraction processes, as enantiomerisation of hyoscyamine from (-) to (+)-hyoscyamine is possible under aqueous alkaline

solution and elevated temperature conditions. Although, it was reported that insignificant quantity is obtainable in breast milk (Alexander *et al.*, 2008), stage of development of the Wistar rats may also be considered as the possible reason of the discrepancy, as adolescence is considered as a critical period of neuronal plasticity, hence easily susceptible to neurotoxic tendencies. Studies have also found that exposure to tropane alkaloids to influence the quality (unpleasant taste) and quantity (yield) of milk from lactating animals (Alexander *et al.*, 2008), and that passage of substances through breastmilk of the lactating mothers to their newborn babies rely on different various factors, which include; physical and chemical properties of the substances, maternal physiology and molecular velocity of substances (Clewett & Gearhart, 2002). By extension, the pups probably did not receive enough dose of the hyoscyamine fraction to cause damage in the hippocampal neurogenesis that could alter the memory. Another reason attributable to this might probably cause by the short exposure duration and rapid metabolism of the alkaloids in the biological system, as it was reported that oral absorption of the anticholinergic agents, such as atropine and glycopyrrolate was poor as a trace or no amount was reported to be found in breast milk (Hale, 1999).

Hippocampus locates under the cerebral cortex and plays an important role in memory formation (Káli & Dayan, 2004) and navigation (Koene *et al.*, 2003). It subdivides into narrow areas with distinguished parts, known as Cornu Ammonis (CA) areas. The cornu ammonis (CA1) neurons are important processing episodic memory in the rats (Bartsch *et al.*, 2011). The current study did not observe serious changes in the histology of CA1 regions of the hippocampus between the groups. However, a slight patch of smaller but hyperchromic nuclei, mild cytoplasmic vacuolations and pyknotic cells were observed in the treated group. These observations indicate changes in the histology of the CA1 region caused by the fraction probably due to the release of generated neurotoxic elements such as reactive oxygen species resulted from the action of the fraction. Studies have confirmed that release of nitric oxide, p53, residual oxygen species and cytokines cause excitotoxicity, which could lead to cells loss in the hippocampus (Coyle and Puttfarcken, 1993; Epstein *et al.*, 1994; Ankarcrona *et al.*, 1995; Morrison *et al.*, 1996). To our knowledge, no similar works of literature offer data about histopathological effects of hyoscyamine fraction of *D. stramonium* seeds on pups via lactation, however, Ekanem *et al.* (2016) reported cytoplasmic vacuolation, cellular necrosis in adult Wistar rats treated with ethanol extract of *D. stramonium* seeds intraperitoneally. Also, Bihaqi *et al.* (2012) reported a neuronal lesion characterized by necrosis, ghost cells, haemorrhage and cytoplasmic vacuolations in rats that received intraperitoneal treatment of scopolamine. All tropane alkaloids of *D. stramonium* Linn species parts have central anticholinergic symptoms as it can cross the blood-brain barrier and cause long-lasting effects (Bania *et al.*, 2004). It induces hypnosis and neuronal degeneration (Hughes & Clark, 1939).

5. Conclusion

In conclusion, exposure of Wistar pups to hyoscyamine fraction of *D. stramonium* L. seeds at lactation causes mild changes in the histoarchitecture of the CA1 region,

a loss in short-term but not enough to impair spatial learning and memory functions of the hippocampus of Wistar rats at adulthood.

Patient informed consent: There is no need for patient informed consent.

Ethics committee approval: The ethics committee approval has been obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2018/042).

Conflict of interest: There is no conflict of interest to declare.

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Author contribution subject and rate:

Idris Abdu Tela (50%): Design the research, data collection and analyses and wrote the whole manuscript.

Sunday Abraham Musa (20%): Organized the research and supervised the article write-up. *Ibrahim Abdullahi Iliya (15%):* Contributed with comments on research design and slides interpretation.

James Oliver Nzalak (15%): Contributed with comments on manuscript organization and write-up.

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