Aqueous extract of Datura stramonium seeds induces cerebellar neurodegeneration, motor and non-motor deficits in Wistar rats

Idowu Olumayowa Kolawole[1]

[1] Department of Anatomy, College of Medicine, University of Lagos.

Corresponding author: Idowu Olumayowa Kolawole (saintmayorfa@yahoo.com).

ABSTRACT

INTRODUCTION: *Datura stramonium* is a psychoactive plant with several active phytochemicals which includes tropane alkaloids such as atropine, scopolamine and hyoscyamine and other phytoconstituents reported to have mind-altering effects. The cerebellum is involved in motor and non-motor functions, consisting of cognition and affective states.

MATERIALS AND METHODS: Twenty (20) rats were blindly randomized into four groups (A, B, C and D; n=5/group). Distilled water (5 mL/kg; p.o) was administered to the control group (A), while 100, 150 and 200 mg/kg (p.o) doses of DSE was administered to the test groups B, C and D respectively for a period of 21 days.

RESULTS: The biometric and behavioral results showed that DSE in a dose-dependent fashion markedly increased percentage body weight gain and number of foot slips but reduced the number of alternation, percentage of alternation and average speed. The neurotoxicity of DSE also showed a dose dependent histomorphological changes in cerebellar cortex.

CONCLUSION: This study shows that DSE decreases locomotion, equilibration and balance, spatial memory, and cognition in treated rats.

KEYWORDS Cerebellar degeneration, Datura stramonium, Motor and non-motor deficits

INTRODUCTION

Datura stramonium (*D. Stramonium*), commonly referred to as thorn apple in English, is an annual and herbaceous plant that belongs to the circle of relatives of solanaceae [1,2]. It is highly rich in alkaloids, tannins, saponins, glycosides, flavonoids, carbohydrates, proteinsand phenolic compounds [3]. Earlier research mentioned that this plant possesses a number of pharmacological properties such as anti-microbial, anti-cholinergic, anti-inflammatory, anti-asthmatic, antiulcer, anti-cancer, analgesic and lot more, used for the remedy of numerous diseases [4]. Nonetheless, the toxicity of this plant especially the tropane alkaloids (atropine, scopolamine and hyosciamine) and other phytoconstituents showed to have adverse effects on the nervous system; causing loss of memory, induction of hallucination, and anxiety [5].

Physical interplay and few clinical cases reports have revealed the purposeful use of D. *Stramonium* amongst adolescents specifically for its central nervous system (CNS) stimulating effects [6]. In addition, instances of unintended *D. Stramonium*-induced toxicity in humans are mostly via consumption of contaminated farm products [7].

The cerebellum (also called “little brain”) is the largest part of the hindbrain anatomically located at the posterior part of the brain, underlying the occipital and temporal lobes of the cerebral cortex [8]. It is dorsal to the pons and medulla, and its median region is separated from them by means of the fourth ventricle [9]. Although the cerebellum accounts for about 10-11% of the brain’s volume, it contains over 50% of the total number of neurons in the brain [10].

The cerebellum is typically regarded for its involvement in motor coordination [11]. However, lines of evidence revealed cerebellum to play significant roles in non-motor activities like emotion, learning, cognition and maintenance [12-14]. Furthermore, cerebellar networks show long-term synaptic plasticity, which indicates that experiment-dependent adaptation and learning processes are also a prominent feature

of cerebellar function [15,16]. The characteristic adaptive capacity is a key feature of many current theories of cerebellar functions [17]. Animal studies showed that cerebellar injuries might cause movement incoordination, difficulty in balance and interrupt learning on cognitive task. Here we evaluated the changes in motor coordination, spatial memory, cognition and cerebellar histomorphology of *D. stramonium*-treated rats using Ymaze and beam walking tests, and H&E histological assessment.

MATERIALS AND METHODS

All experimental procedures were strictly carried out in conformity with the "Guidelines for Care and Use of Laboratory Animals" prepared by the National Academy of Science and sanctioned by the Ethical Research Committee of the University of Ilorin, Nigeria.

Morphometric measurements

The sample of *D. stramonium* seeds *were* harvested from the botanical garden of the University of Ilorin, Nigeria. Botanical identification was done at the Department of Plant Biology, Faculty of Physical Sciences, University of Ilorin, Nigeria.

The extraction was carried out according to the method of Fatoba *et al.*, 2012 with modifications. The seed pods were plucked, left for three days to crack before the seeds were collected. Subsequently, the seeds were air-dried at room temperature and grounded into fine powder. 500g of the fine powder was soaked in 10L of distilled water for three days to extract its constituents. The preparation was then filtered and the residue was discarded while the filtrate was evaporated to dryness over the oven at a temperature of 60–80°C for 5-6 hours per day for 2 weeks. *D. stramonium* solution of 0.1g per ml concentration was prepared, kept in a freezer (-20ºC) to avoid fermentation and deterioration [18]. The lethal dose (LD_{50}) of *D. stramonium* seed extract was 400 mg per kg body weight [19]. In this study, the extract doses range between ¼ and ½ of the lethal dose which are 100, 150 and 200 mg/kg body weight, administered to groups B, C and D respectively.

Experimental animals

Adult female Wistar rats (20) weighing 200±20 g were obtained from the animal holding of the University of Ilorin, Nigeria, kept and observed for general well-being in the animal house the Department of Anatomy, University of Ilorin. They were housed in plastic cages, maintained under standardized conditions (12-h light/dark cycle, 27-30º C, 50-80% relative humidity), fed with standard rodent pelleted diet (Ace Feeds, Ilorin, Nigeria) and water *ad libitum*. The rats were acclimatized in the laboratory for 14 days before the commencement of the study [20].

Experimental design

The rats were randomly divided into two groups; the control (n=5) and test (n=15) groups. The control group (A) received distilled water (5 mL/kg) while the test group was further divided into three subgroups (n=5/subgroup; B, C and D), which received DSE at varying doses of 100, 150, and 200 mg/kg body weight respectively (Figure 1). Both distilled water and DSE were administered via intra-gastric gavage daily for 3 weeks.

Sub-acute neurobehavioral assessments

The rats were subjected to neurobehavioural tests on 14th and 20th day of the administration in order to access motor cognitive functions (Figure 1). The Y-maze and beam walking tests were carried out on the 14th and 20th day respectively after three consecutive trials for a period of 3 days per test. The Y-maze test was used to evaluate the effect of DSE on spatial cognitive ability of the rodents. The apparatus comprised three arms (75 cm × 25 cm × 15 cm) labeled A, B and C, which are symmetrically separated at 120°. The procedure involved the placement of individual rat in the Y-maze apparatus at the end of arm A, allowed it to explore all the three arms freely for 5 minutes and recorded the number of arm visits and alternation of arm visits visually [21]. Sequence such as ABC, BCA or CAB was identified as correct alternation; the percentage alternation is used as an index of spatial memory which was calculated as shown below:

$$\% \text{ Alternation} = \frac{\text{Number of right decisions}}{\text{Number of total arm entries - 2}} \times 100$$

The beam walking test is an experimental measure used to assess the balance and motor coordination of the rodents under the influence of drug treatment [22]. In this study, individual rat was placed on a white, brightly lit platform and was allowed to walk across a narrow wood beam to reach an enclosed safety dark box end of the beam with pelleted diet inside. To evaluate the motor coordination and balance, time taken by the rat to traverse the distance; number of foot slips and turns that occurred during the process were recorded. Average speed was calculated as the rate of distance covered to traverse the beam per unit time, expressed in cm/s.

Measurement of body weight

The rats were weighed with the electrical balance at day 1 and 21 prior to the first administration and the sacrifice of the rats respectively. The percentage of weight gain per rat was calculated using the following arithmetical equation:

$$\% \text{ Weight gain} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Histomorphological study

At day 21, the rats were euthanized by intra-peritoneal injection of 0.2ml/100g of ketamine and intracardially perfused with 0.9% normal saline followed by 4% paraformaldehyde (PFA). Brain tissues were excised, rinsed in 0.25 M sucrose 3 times for 5 min each and post-fixed in

10% phosphate-buffer formalin for five days. Thereafter, cerebellar tissues were sectioned, mounted and stained with Haematoxylin and Eosin (H and E) as described by Imam et al. (2016) for histological evaluation [23]. Specimens were examined under a light microscope (magnification, ×200).

Data analysis

Statistical evaluations were done using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Behavioural and body weight groups of data were compared with an analysis of variance (ANOVA) followed by Turkey multiple comparison tests. All data are expressed as mean ± S.E.M. Analysis was statistically significant at a two tailed p value of < 0.05.

RESULTS

Aqueous extract of Datura stramonium seeds (DSE) enhances body weight gain in rats

The effect of DSE on body weight gain is illustrated in Figure 2A. The percentage body weight gain shows that there was significant increase (p < 0.05) in body weight of rats that received DSE (150 and 200 mg/kg) when compared to control rats. There was no significant difference (p > 0.05) in body weight of rats that received 100mg/kg DSE when compared to control rats.

Aqueous extract of Datura stramonium seeds (DSE) reduces spatial memory and locomotion performance of rats in Y-maze

The effect of DSE on the number and percentage of alternation in rats subjected to Y-maze test is shown in Figures 2B and 2C respectively. DSE (150 and 200 mg/kg) treatment caused disruption in locomotion by significantly decreasing (p < 0.05) the number of alternations while the exposure of 100 mg/kg DSE showed no effect (p >0.05) on locomotion when compared to rats that received distilled water. Treatment with all doses of DSE significantly reduced (p < 0.05) the sequence of percentage alternations when compared to distilled water treated rats. The result of Y-maze test showed deficits in locomotion, spatial working memory or the readiness of rat to explore a new terrain when treated with DSE.

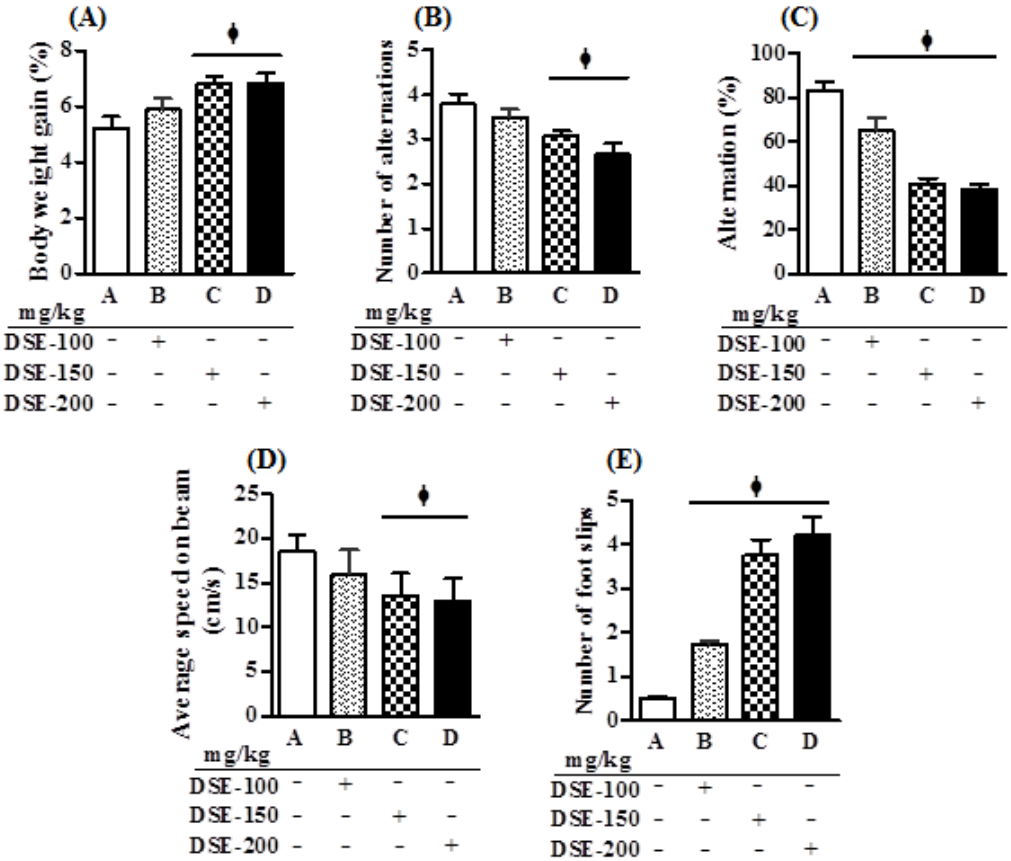

Figure 2. Aqueous extract of Datura stramonium seed enhances body weight gain, and impairs motor-cognitive functions in female Wistar rats as depicted by: A) percentage body weight gain, B) percentage spontaneous alternation, C) average speed on beam and D) numbers of foot slips. DSE exposure significantly increases body weight gain. The results were expressed as the mean ± S.E.M. (n=5), ΦP <0.05 versus the control group.

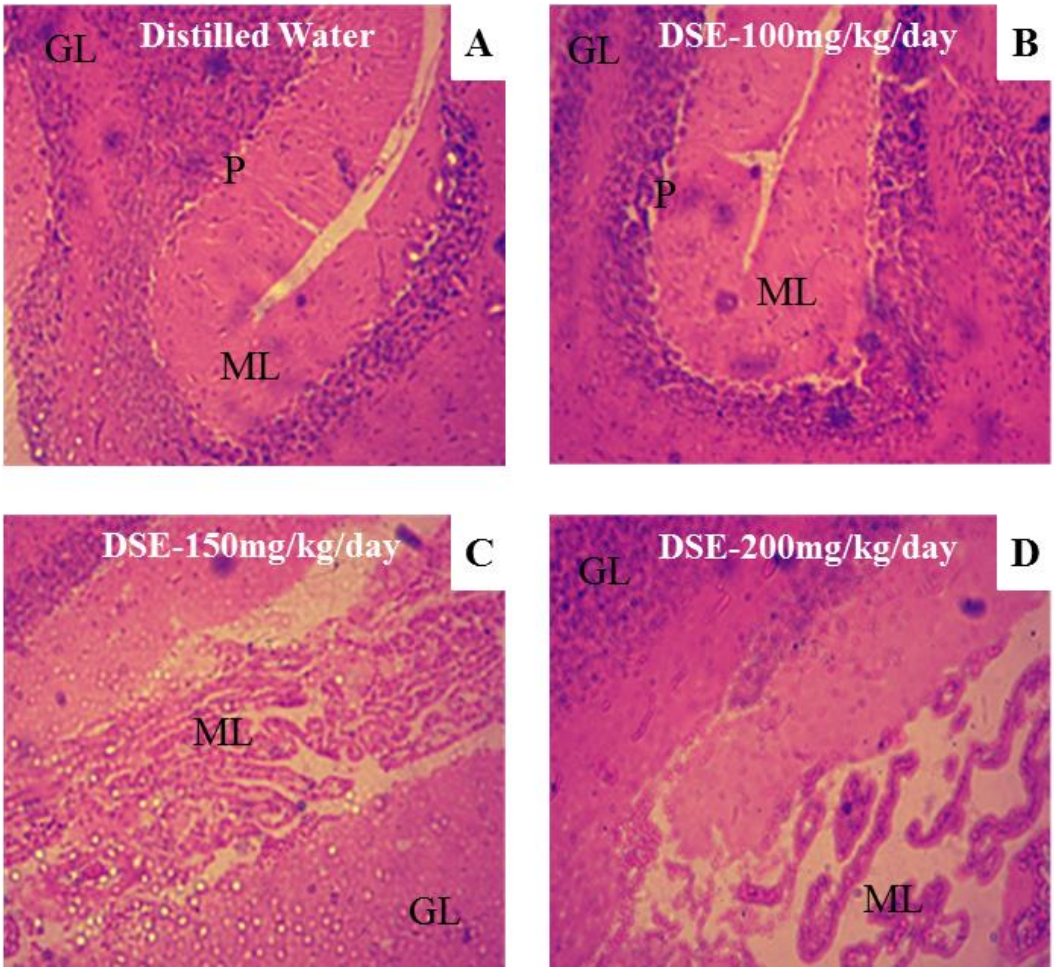

Figure 3. Representative photomicrograph: Aqueous extract of *Datura stramonium seed* (DSE) deranges cerebellar histoarchitecture in a dose graded pattern. Rats in the following groups received (A) Distilled water, and shows normal histomorphology (B) DSE-100 mg/kg, shows normal histomorphology with no visible change cellular arrangement. (C) DSE-150 mg/kg, formation of vacuoles, loss of cells in Purkinje layer and (D) DSE 200 mg/kg, disruption of cellular organization in the cerebellar cortex. P: Purkinje; ML: molecular layer; GL: granular layer; stain: hematoxylin and eosin; magnification: x200

Aqueous extract of Datura stramonium seeds (DSE) alters performance of rats in beam walking test

The effect of DSE on average speed of rats away from the beam and the number of foot slips in rats subjected to beam walking test is shown in Figures 2D and 2E respectively. Treatment of rats with DSE significantly decreased ($p < 0.05$) the average speed on beam except 100 mg/kg DSE that had no significant change ($p > 0.05$) when compared to distilled water treated rats. In addition, all the DSE treated rats (100, 150 and 200 mg/kg) showed significant increase in the number of foot slips ($p < 0.05$) which affected the balance pattern when compared with distilled water treated rats. The result of beam walking test showed impairments of balance and motor coordination in DSE treated rats.

Aqueous extract of Datura stramonium seed (DSE) alters cerebellar histoarchitecture in rats

The effects of DSE on cerebellar tissue were observed to be toxic in a dose graded patterns as shown in Figure 3A-D. The histomorphological examination of the cerebellum in control rats that received distilled water showed normal histoarchitecture as the arrangement of the three cerebellar layers and neuronal morphology were all intact (A). 100 mg/kg DSE showed no visible change in the Purkinje cell layer and retained normal arrangement (B). Meanwhile, DSE at 150 mg/kg led to atrophy of Purkinje cell layer and shrunken nuclei of the neurons in the molecular layer with disruption of fibers orientation while in granular layer, there was formation of vacuoles in the cells of the cerebellum suggesting degeneration of the nerve cells (C). Furthermore, DSE at 200 mg/kg caused total distortion of the cellular arrangement in molecular and granular layers, indicating neuronal degeneration (D). The histopathological result of this study showed that DSE at 150 and 200 mg/kg provoked cerebellar degeneration.

DISCUSSION

D. stramonium is a hallucinogenic plant of the family solanaceae, widely found in city and rural areas and commonly

used in alcoholic beverage due to its pharmacological properties [4,24]. It contains a variety of phytochemicals that are worthwhile in the treatment of certain diseases [25]. Notwithstanding its medicinal usefulness, its toxicity at low dosage keeps putting the user at an awesome risk.

After a sub-acute oral administration of 100, 150 and 200 mg/kg doses of DSE, there were remarkable changes in both physical and structural assessment respectively. DSE increased weight gain across the treated groups. This is similar to the report of Uddin *et al*., 2017 where gain in weight was observed at the lowest dose (50 mg/kg) but reduces with higher doses (100 mg/kg and 200 mg/kg) respectively [23]; when solvated DS leaf extracts were administered to evaluate its toxic effect in rats. The underlying mechanism behind the weight gained by the rats is unclear. Perhaps, DS may have stimulating impact on appetite centre of hypothalamus thereby increasing the feeding dependency of rats.

The locomotor activity and spatial memory decreased across the groups in a dose dependent manner. As observed in the total number of alternation and percentage alternation assessments on Y-maze test, the result obtained from these behavioral tests shows that DSE caused impairment of locomotion, memory and cognitive function in the treated rats. This observation accord with the report of Tijani *et al*. (2015), whose study revealed the neuro-toxicological consequences of *Datural metel*; another plant of the family solanaceae with a common active secondary metabolites (atropine and scopolamine respectively). *Datural metel* caused motor dysfunction, reduced total locomotive activity and induced memory deficit in treated mice [26].

Furthermore, the cerebellum is termed the head of ganglion of the proprioceptive system as it receives general impulses from the limbs and trunk through the spinocerebellar tract and accessory cuneate nuclei while the special impulses from semicircular canal system is through the vestibular nerve [27,28]. Upon information provided by these impulses the cerebellum is competent to exert a controlling influence on posture and by regulation of muscular tone to effect the harmonious coordination of all individual muscles required for the production of voluntary movement [29,30]. Thus, the possible explanations for the observation are: lack of adequate input from afferent impulses to cerebellum; interruption of signaling pathway(s) to the cerebellum; defect in vestibular nerve transmission or disturbance in semicircular canal; injury or inflammatory responses along the tracts; impaired integrity of neuronal membrane and substantial neuronal derangement and loss (supported by histomorphological changes figures 3C and 3D).

From the beam walking test, the locomotor activity also decreased across the groups (examined by average speed of rats on beam), whilst the balance was altered (examined by number of foot slips) in a dose dependent fashion. This result shows that DS aqueous extract causes significant lack of muscle coordination, the motor muscle coordination deficiency of these rats could be ascribed primarily to the dysfunctions of the cerebellar Purkinje cell synapse formation. The Purkinje cells are the exclusive efferent neurons of the cerebellar cortex among other four cells (molecular layer, Golgi cell, Mossy fibre and granule cells) and essential to cerebellar cortical information processing [31,32].

Interestingly, the histological studies revealed the progressive loss of normal arrangement of cellular layers and atrophy of cell bodies in Purkinje layer of the cerebellum. The rats treated with 150 mg/kg and 200 mg/kg of aqueous extract of DS seeds displayed moderate to severe histoarchitectural distortion, substantial cell death and neuronal degeneration. This neurodegeneration may be due to interference involving a cascade of neurochemical signaling pathways in the cerebellum [33,34], evidenced by loss of Purkinje layer, the only efferent pathway of cerebellum.

CONCLUSION

Sustained oral administration of aqueous extract of *D. stramonium* seeds, based on the dosage used in this study, increased body weight, but decreased locomotion, spatial memory and cognition. It also caused changes in cerebellar histoarchitecture, as well as disturbances of equilibration and balance (ataxia) in treated animal model in a dose dependent manner.

REFERENCES

1. Al-Snafi AE. Medical importance of Datura fastuosa (syn: Datura metel) and Datura stramonium.A review. IOSR*Journal of Pharmacy*. 2017 Feb 7(2):43-58.
2. Fatur K, Kreft Common anticholinergic solanaceaous plants of temperate Europe-A review of intoxications from the literature (1966–2018). *Toxicon*. 2020 Apr 15; 177:52-88.
3. Benítez G, March-Salas M, Villa-Kamel A, Cháves-Jiménez U, Hernández J, Montes-Osuna N, Moreno-Chocano J, Cariñanos P. The genus Datura L. (Solanaceae) in Mexico and Spain–Ethnobotanical perspective at the interface of medical and illicit uses. *Journal of ethnopharmacology*. 2018 Jun 12; 219:133-51.
4. Cunningham, N. Hallucinogenic plants of abuse. *Emergency Medicine Australasia*. 2008 Apr 20(2):167-74.
5. Velu G, Palanichamy V, Rajan AP. Phytochemical and Pharmacological Importance of Plant Secondary Metabolites in Modern Medicine. *Bioorganic Phase in Natural Food: An Overview*. 2018 Apr 20:135.
6. Adegoke S, Alo L. Datura stramonium poisoning in children. *Nigerian Journal of Clinical Practice*. 2013 Jan 1;16(1):116-116.
7. Ademiluyi AO, Ogunsuyi OB, Oboh G. Alkaloid extracts from Jimson weed (Datura stramonium L.) modulate purinergic enzymes in rat brain. *Neurotoxicology*. 2016 Sep 1; 56:107-17.
8. Newton BW. Anatomy of the Spinal Cord and Brain. *Neuroscience in Medicine*. 2008:25-51.
9. Goel P, Singla M, Ghai R, Jain S, Budhiraja V, Babu CR. Transverse cerebellar diameter-a marker for estimation of gestational age. *J. Anat. Soc. India*. 2010 Dec 1;59(2):158-61.
10. Ramnani N. The primate cortico-cerebellar system: anatomy and function. *Nature reviews neuroscience*. 2006 Jul 7(7):511-22.

11. Koziol LF, Budding D, Andreasen N, D"Arrigo S, Bulgheroni S, Imamizu H, Ito M, Manto M, Marvel C, Parker K, Pezzulo G. Consensus paper: the cerebellum's role in movement and cognition. *The Cerebellum*. 2014 Feb 1;13(1):151-77.

12. Zhu JN, Yung WH, Kwok-Chong CB, Chan YS, Wang JJ. The cerebellar-hypothalamic circuits: potential pathways underlying cerebellar involvement in somatic-visceral integration. *Brain research reviews*. 2006 Aug 30;52(1):93.

13. Adamaszek M, D'Agata F, Ferrucci R, Habas C, Keulen S, Kirkby KC, Leggio M, Mariën P, Molinari M, Moulton E, Orsi L. Consensus paper: cerebellum and emotion. *The Cerebellum*. 2017 Apr 1;16(2):552-76.

14. Sathyanesan A, Zhou J, Scafidi J, Heck DH, Sillitoe RV, Gallo V. Emerging connections between cerebellar development, behaviour and complex brain disorders.

15. Ito M. ITO M. Cerebellar Long-Term Depression: Characterization, Signal Transduction, and Functional Roles. *Physiological Reviews*. 2001 Jul 1;81(3):1143-95.

16. Hansel C, Linden DJ, D'Angelo E. Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nature neuroscience*. 2001 May 4(5):467-75.

17. Apps R, Garwicz M. Anatomical and physiological foundations of cerebellar information processing. *Nature Reviews Neuroscience*. 2005 Apr 6(4):297-311.

18. Fatoba TA, Adeloye AA, Soladoye AO. Effect of aqueous extract of Datura stramonium on spermatogenesis of West African Dwarf bucks. *Euro J Zool Res*, 2012, 1 (3):77-79.

19. Antov G, Zaikov C, Bouzidi A, Mitova S, Michaelova A, Halkova J, Choumkov N. Biochemical and histological changes after acute oral poisoning with the acetanilide herbicide acetochlor. *Journal de toxicologie clinique et expérimentale*. 1991 Oct 11(6):349-56.

20. Olayaki LA, Alagbonsi IA, Abdulrahim AH, Adeyemi WJ, Bakare M, Omeiza N. Melatonin prevents and ameliorates lead-induced gonadotoxicity through antioxidative and hormonal mechanisms. *Toxicology and industrial health*. 2018 Sep 34(9):596-608.

21. Abdulmajeed WI, Sulieman HB, Zubayr MO, Imam A, Amin A, Biliaminu SA, Oyewole LA, Owoyele BV. Honey prevents neurobehavioural deficit and oxidative stress induced by lead acetate exposure in male wistar rats-a preliminary study. *Metabolic brain disease*. 2016 Feb 1;31(1):37-44.

22. Jamwal S, Singh S, Bansal PK. Behavioral Tests for Rodent Models. Animal Models of Neurological Disorders: *Principle and Working Procedure for Animal Models of Neurological Disorders*. 2018 Jan 17:315.

23. Imam A, Ajao MS, Amin A, Abdulmajeed WI, Ibrahim A, Olajide OJ, Ajibola MI, Alli-Oluwafuyi A, Balogun WG. Cannabis-induced moto-cognitive dysfunction in wistar rats: ameliorative efficacy of nigella sativa. *The Malaysian journal of medical sciences: MJMS*. 2016 Sep 23(5):17.

24. Uddin F, Hossain A, Das R, Rahman Ahmad M, Akanda R, Islam S. Evaluation of toxic effects of Datura Leaves (Datura stramonium) in rat. *Int. J. Agric. Environ. Res*. 2017;3(4):3486-97.

25. Naik V, Babu KS, Latha J, Kolluru B. A review on Phytochemical and Pharmacological activity of DaturaStramonium.(Medicinal Plant). *Research Journal of Pharmacognosy and Phytochemistry*. 2018 Jul 10(1):77-80.

26. Tijani A, Eyineyi U, Ibrahim J, Okhale S. Neurotoxicological impacts of datura metel linn.(family: solanaceae) leaves extract in mice. *The Journal of Neurobehavioral Sciences*. 2015 Oct 2(3):97101.

27. Hahn C, Masty J. Overview of Neuroanatomy. *Equine Neurology*. 2015 Apr 16:1-20.

28. Tanabe HC, Kubo D, Hasegawa K, Kochiyama T, Kondo O. 18 Cerebellum: Anatomy, Physiology, Function, and Evolution. *Digital Endocasts: From Skulls to Brains*. 2017 Dec 28:275.

29. Sokolov AA, Miall RC, Ivry RB. The cerebellum: adaptive prediction for movement and cognition. *Trends in cognitive sciences*. 2017 May 1;21(5):313-32.

30. Lawrenson C, Bares M, Kamondi A, Kovács A, Lumb B, Apps R, Filip P, Manto M. The mystery of the cerebellum: clues from experimental and clinical observations. *Cerebellum & ataxias*. 2018 Dec 5(1):8.

31. Maex R, Schutter ED. Synchronization of Golgi and granule cell firing in a detailed network model of the cerebellar granule cell layer. *Journal of neurophysiology*. 1998 Nov 1;80(5):2521-37.

32. Van Kan PL, Gibson AR, Houk JC. Movement-related inputs to intermediate cerebellum of the monkey. *Journal of neurophysiology*. 1993 Jan 1;69(1):74-94.

33. Matilla-Dueñas A, Ashizawa T, Brice A, Magri S, McFarland KN, Pandolfo M, Pulst SM, Riess O, Rubinsztein DC, Schmidt J, Schmidt T. Consensus paper: pathological mechanisms underlying neurodegeneration in spinocerebellar ataxias. *The Cerebellum*. 2014 Apr 1;13(2):269-302.

34. Lalonde R, Strazielle C. Spontaneous and induced mouse mutations with cerebellar dysfunctions: behavior and neurochemistry. *Brain research*. 2007 Apr 6; 1140:51-74.