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Full-Length Research Article

Microscopic Assessment of the Effects of *Cannabis Sativa* Leaf Extract on the Cerebellum in Male Wistar Rats

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Summary: Cannabis remains the most abused illicit substance in the world. The growing inclination towards decriminalisation in several countries creates the need to determine how consumption affects the organs of the body, especially the brain. This research aims to determine the effect of *cannabis sativa* on the histoarchitecture of the cerebellum and motor coordination in Wistar rats. Twenty-five male rats were divided into 5 groups (I-V): Group I received food and water, Group II received 10 mg/kg, and Group III received 20mg/kg of *cannabis sativa* aqueous leaf extract for 28 days. Groups IV & V received 10 mg/kg and 20 mg/kg of *cannabis sativa* for 28 days and were allowed for another 28 days recovery period. At the end, animals were weighed and sacrificed by cervical dislocation. Results from Transmission Electron Microscope (TEM) sections of rat cerebellum in group I show normal neurons and cellular architecture. Some degrees of cytoarchitectural and neuronal distortions were observed in animals exposed to cannabis. The extent of these distortions, however, was significantly reduced following a 28-day recovery period for both doses of cannabis administered. It can be deduced, therefore, that *cannabis sativa* exposure had significant adverse ultrastructural effects on the cerebellum of adult male Wistar rats.

Keywords: *Cerebellum, transmission electron microscope (TEM), cannabis sativa, ultrastructure, histoarchitecture*

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INTRODUCTION

Cannabis sativa is a commonly abused plant due to its high content of the psychoactive compound, THC (Lucas et al., 2018; Freels et al., 2020). Though cannabis has been used for medical purposes due to its antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective properties, its adverse consequences should not be underestimated (Ford et al., 2017; Bloomfield et al., 2019). Despite reported claims of cannabinoids (CBD), a non-psychoactive constituent of *Cannabis sativa* having some neuroprotective ability, inhibiting neurodegeneration, and as a promising agent in several neurodegenerative diseases (Cassano et al., 2020), there is need to clearly define its benefits and its harmful effects.

Cannabis sativa is an annual herbaceous flowering plant indigenous to Eastern Asia, but now of cosmopolitan distribution due to widespread cultivation. It has been cultivated throughout recorded history, used as a source of industrial fiber, seed oil, food, recreation, religious and spiritual moods and medicine. Each part of the plant is harvested differently, depending on the purpose of its use. The species was first classified by Carl Linnaeus in (1739).

Cannabis also produces numerous volatile sulfur compounds that contribute to the plant's skunk like aroma, with Prenylthiol (3-methyl-2-butene-1-thiol) identified as the primary odorant (Oswald et al., 2021). The active component of the marijuana plant *Cannabis sativa* was first identified as Δ9-tetrahydrocannabinol (THC) (Malabadi et al., 2023). THC, also known as dronabinol, produces numerous beneficial effects, including analgesia, appetite stimulation, nausea reduction and reduction of intraocular pressure (IOP). THC also affects bone remodeling, fertility, short term memory, tumor growth and motor coordination (Smith et al., 2020; Mechoulam, 2012; Iversen, 2005).

Cannabis use is common among adolescents and young adults, but the long-term consequences of such use are a topic of debate. Cannabis use typically starts during early adolescence and peaks when users are in their mid-20s (Hall et al., 2020; Hasin 2018). Cannabis use can have adverse health effects, including increased risks for lung, cardiovascular, and periodontal diseases (Gordon et al. 2013; Jouanjus et al. 2017; Lorenzetti et al., 2019). Its effects on development of cognitive and affective dysfunction, however, have been less conclusive. An initial study reported that cannabis use, particularly during adolescence, contributes to a lasting neurocognitive decline including an 8- point drop in IQ from childhood to adulthood (Chye et al., 2020; Jackson et al. 2016). More recent studies, however, do not support this conclusion.

Receptors for THC and other cannabinoid compounds are present in the brain, especially in the frontal cortex, basal ganglia, cerebellum, and limbic regions. Cannabinoid

action in the basal ganglia and cerebellum probably account for the effect on psychomotor control (Ashton et al., 2017). The cerebellum has an important role in motor control, with cerebellar dysfunction often presenting with motor signs. It is active in the coordination, precision, and timing of movements, as well as in motor learning (Figueiredo et al., 2020; Lorenzetti et., al 2010). They reported neuronal alterations in chronic cannabis use can lead to structural changes in the cerebellum. One of the most prominent effects is the reduction in the size and complexity of Purkinje cells, which are the principal neurons in the cerebellum. These changes may be associated with impaired motor coordination and balance in chronic users. Synaptic Changes because of cannabis impact can cause synaptic plasticity in the cerebellum, thereby affecting the integrity and efficiency of neuronal connections. This is crucial for motor learning and coordination (Battistella et al. 2014).

In a study of the combined and independent effects of chronic cannabis use and HIV on brain metabolites, Chang et al. (2006) found that cannabis use was associated with a decrease in neuronal and glial metabolites, yet a normalization of glutamate levels in PWH (Chang et al., 2006; Bahji et al., 2021). Adult studies of marijuana use often find subtle decreases in performance compared to controls in cognitive domains such as attention, memory, and processing speed; such effects have been discussed as transient in literature given limited group differences after prolonged abstinence from marijuana (Grant et al, 2003; Pope et al, 2010; Krist et al., 2020).

In contemporary research, molecular studies take central stage in biomedical research (Hindocha et al., 2020). Ultrastructural studies provide the best option available to explore histological structures, hence its adoption in this study.

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MATERIALS AND METHODS

Ethical approval: Ethical approval for this research was sought and obtained from the research ethics committee (REC) of Nnamdi Azikiwe University Awka Nnewi campus.

Animals: Rats were obtained from the Animal house of the College of Medicine and Health Sciences, Nnamdi Azikiwe University Nnewi Campus. The animals were housed within the standard facilities of a well-ventilated animal house and maintained on pelletized rat chow and water ad libitum under standard laboratory conditions of lighting and moderate temperature.

Twenty-five male rats were divided into 5 groups (I-V): Group I received food and water, Group II received 10mg/kg and Group III received 20mg/kg of cannabis sativa aqueous leaf extract for 28 days. Groups IV & V received 10mg/kg and 20mg/kg of cannabis sativa for 28 days and were allowed for another 28 days recovery period. Plant material: Fresh leaves of Cannabis sativa were obtained from the Locals. The plant was authenticated by Mr. Iroka Finian, a taxonomist at the Nnamdi Azikiwe University Herbarium, Awka, Anambra state. Yadima et al., (2017) modified method of Silva & Afkinnghorn, (1988) standard method was used for aqueous extraction. The plant material was soaked in distilled water for 24 hours and vigorously shaken intermittently. Mixture was then evaporated using a water bathe until a gummy brown deposit is formed. This was labelled and refrigerated until use. Stock solution of the extract was constituted by dissolving 1g of the extract in 10ml of distilled water.

Tissue processing: For tissue processing for H&E, small portions of rat cerebellum was cut and fixed in 10% neutral buffered formalin. After 48 hours of fixation, tissues were processed using the H&E procedure according to the method of Carleton et al., (1967). Tissues were placed in ascending grades of alcohol (60%, 70%, 80%, 90%) for one hour each and in absolute (100%) twice, one hour each. Tissues were immersed in two changes of xylene for one hour each, infiltrated in four changes of molten paraffin wax at constant temperatures of 36-40ºC in an oven of paraffin bath for one hour each and embedded with Metal blocks. Thin sections were made at 5µm using a rotary microtome, and picked up with a prelabelled glass slide made stick using egg albumin. Haematoxylin and eosin staining was done according to the procedure described by Carleton et al., (1967) and mounted in distrene plasticizer xylene (DPX) using clean glass cover slide. Tissues were then focused under Leica research light microscope and photomicrographs were taken from each group and labelled using Microsoft PowerPoint.

As regards tissue processing for Electron Microscopy, small pieces of rat cerebellar tissue were fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.4 followed by a wash in phosphate buffer, pH 7.4 for 2 hours. Tissues were immersed and postfixed for 1 hour in 1% osmium tetroxide and then placed in 70% alcohol overnight in the refrigerator at 4°C. Tissues were dehydrated through 2 changes of 95% alcohol and absolute alcohol for 20 minutes, cleared, and infiltrated with propylene oxide and Epon-Araldite resin solutions of varying ratios. First, in a solution of 3 parts propylene oxide to 1 part resin, second, in equal parts propylene oxide and resin solution, and third in a solution of 1 part propylene oxide to 3 parts resin, for a length of 30 minutes per solution. Lastly, the tissues were left overnight in resin, followed by embedding in fresh Epon-Araldite resin at 60∘C for 48 hours (Goodhew, 2011).

After polymerization, 1 μm sections were cut on a Reichert-Jung Ultracut ultra microtome (Germany) and stained with Toluidine Blue-Pyronin Y for 30 seconds, dried, and mounted in Entellan. Ultrathin gold sections were cut and placed on copper grids and stained with uranyl acetate for 3 minutes. Drops of lead citrate were placed on strips of dental wax, and once stained, grids were rinsed first in dilute sodium hydroxide, followed by distilled water and then dried (Goodhew, 2011).

The work on electron microscopy was carried out at the Noguchi Memorial Institute for Medical Research, Ghana.

RESULTS

Haematoxylin & Eosin Light Microscopy: The results for light microscopy of rat cerebellum are presented in plate 1 (L1 to L5 for rat in groups I to V respectively). The control group (L1) show typical histological features of the cerebellum. There are three distinct layers; granular layer (GL), Purkinje cell layer (PCL) and molecular layer (ML). The Purkinje layers are composed of large Purkinje neurons with large round nucleus, and prominent nucleoli with glial

cells interspersed within the neurons. The micrograph of rat cerebellum from the treated groups (L2 to L5) show no signs of tissue alteration when compared to the control group.

Electron microscopy: The micrographs of rat cerebellum are presented in plates designated EM 1 to EM5 for rats in groups I to V respectively. Each group is represented by four different micrographs designated (a to d) to show various aspects of the cerebellar ultrastructure from each group.

The ultrastructural investigation of brain tissue of control group showed normal histoarchitecture of the cerebellum under the Transmission Electron Microscope (TEM). The slides of rat cerebellum from Group I (EM 1a-d) showed normal nucleus with evenly distributed chromatin materials, presence of nucleoli and dense double layered membrane. Mitochondria in the control group exhibited homogenous dense matrix, double membrane integrity with organized cristae. Myelin sheath is thick, electron dense and tightly wrapped around their axon with good synaptic complex, presenting normal thick electron dense tightly wrapped myelin sheath.

In the test Group II (EM 2 a - d) treated with 10mg/kg body weight, TEM showed degenerative chromatin material, absence of nucleus with crescent formation, degenerated cristae, myelin sheet degeneration and no synaptic complex.

In test Group III (EM 3 a - d) treated with 20mg/kg body weight, TEM showed pyknotic nucleus, degenerated chromatin material with distorted nuclear membrane, crescent formation, degenerated cristae, disconnected myelin sheet and no synapse.

The ultrastructure of group IV rat cerebellum under the TEM (EM 4 a - d) showed some degree of recovery in form of double layer nuclear membrane, presence of nucleoli and chromatin material remarkably, close to normal mitochondria cristae and myelin sheath, with good number of synaptic complexes. In the High dose recovery group (Group V) treated with 20mg/kg, TEM (EM 5 a - c) the EM showed degenerated chromatin materials, close to normal nucleoli and broken nuclear membrane. It also showed close to normal mitochondrial integrity with visible cristae, bulged and disconnected myelin sheath with remarkable synaptic complex (Plate EM 5a-c).

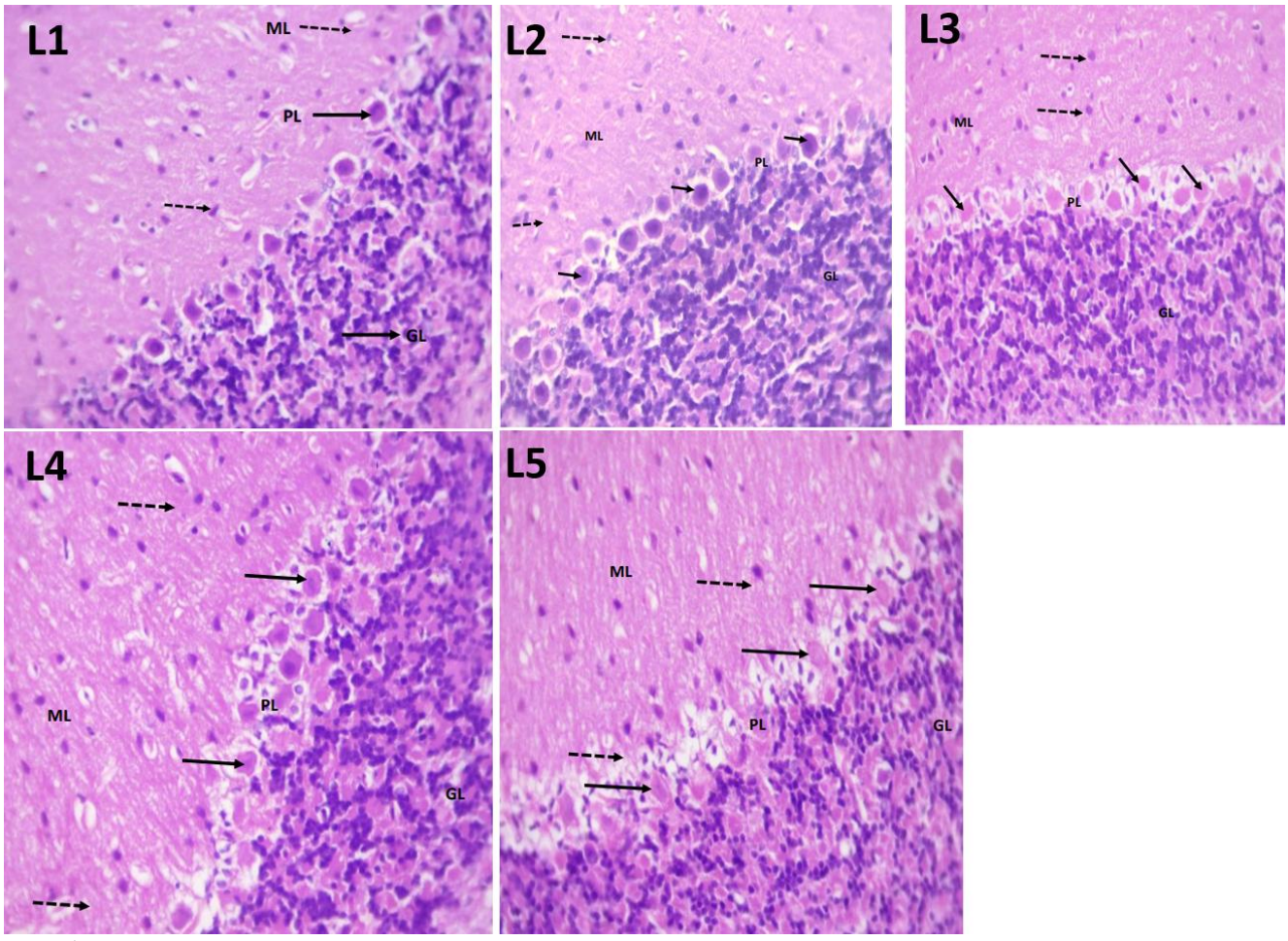

Plate 1

Photomicrograph of section of the cerebellum of rats treated with vehicle (control, L1), 10mg/kg (L2) and 20mg/kg (L3) of *cannabis sativa* for 28 days. L4 and L5 are photomicrographs of the cerebellum of 10mg/kg amd 20mg/kg of *cannabis sativa* treated rats with additional 28-day recovery period respectively..

ML – molecular layer; PL – purkinje layer; GL – granular layer; black arrows – intact purkinje neurons; dashed arrows – glial cells. H&E x400 magnification

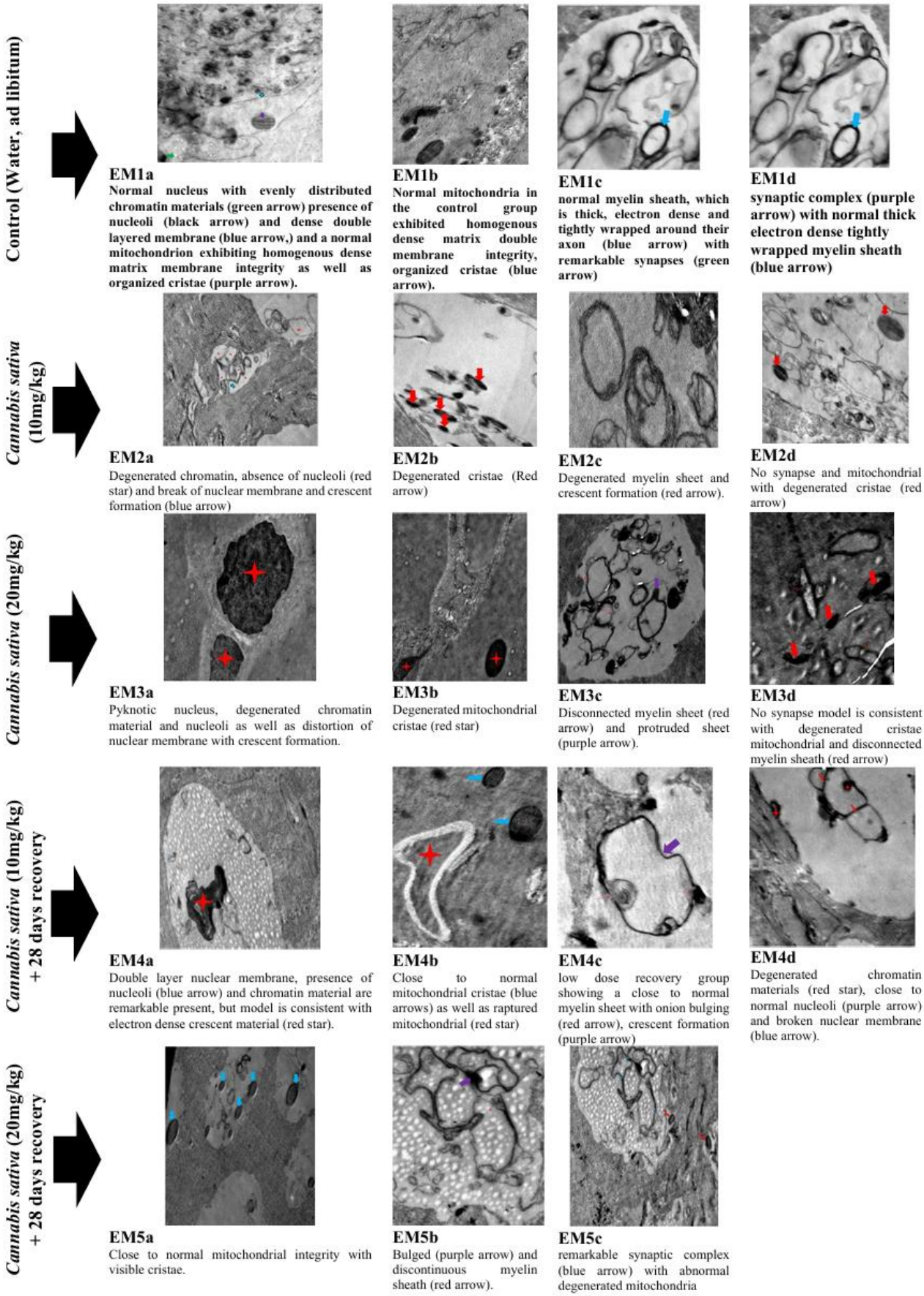

Plate 2
TEM Photomicrograph of section of the Cerebellum of Wistar rats treated with water (EM1a-d), 10mg/kg Cannnabis sativa (EM2a-d), 20mg/kg Cannnabis sativa (EM3a-d). EM4a-d and EM5a-c are TEM photomicrographs of the cerebellum of 10mg/kg and 20mg/kg of cannabis sativa treated rats with additional 28-day recovery period respectively.

DISCUSSION

The results from this study agree with the report of Battistella et al., (2014) which shows that cannabis led to synaptic changes, including synaptic plasticity in the cerebellum which affected the integrity and efficiency of neuronal connections. De Faria et al., (2021) report was consistent with this studies on myelin sheath disruption potentially affecting efficient neural communication in the cerebellum. Cannabis may disrupt myelin formation and maintenance, potentially affecting the ultrastructure of the cerebellum and reduces conduction velocity which may alters timing within neural circuits and osculation. (Pajavic et. al., 2014; Almeida and Iyons, 2017). Mild myelin abnormalities are thought to be a primary cause of behavioral alteration (Poggi et.al., 2016). This report is in line with the findings of this study.

The protocol went further to allow animals in groups IV and V treated with 10mg/kg and 20mg/kg respectively just like groups II and III another 28 days recovery period. The ultrastructure of group IV rat cerebellum under the TEM (EM IV a - d) showed some degree of recovery in form of double layer nuclear membrane, presence of nucleoli and chromatin material remarkably, close to normal mitochondria cristae and myelin sheath, with good number of synaptic complexes. Moreover, in the High dose recovery group (Group V) treated with 20mg/kg, TEM (EM V a - d) showed degenerated chromatin materials, close to normal nucleoli and broken nuclear membrane. It also showed close to normal mitochondrial integrity with visible cristae, bulged and disconnected myelin sheath with remarkable synaptic complex (Plate 5). This result throws light on the power of the natural recovery process.

A lot of cellular damage is restored naturally without treatment simply by elimination or withdrawal of the toxicant, including neurons. It therefore poses a question on the long age knowledge that neurons once damaged cannot be recovered or repaired. Removing the offending substance can lead to clinical improvement and, given the available specific treatment for some toxins, early and correct identification of the relevant toxin is important. Neurologists should consider screening for exposure to appropriate neurotoxins according to the neuropathy phenotype (Smyth et al., 2023).

Researchers have shown (Harclerode 1980) that THC could alter mitochondrial shape and induce swelling in several different tissue dependent on the dose (Whan et. al., 2006). This agrees with the findings of this research. Sarafian et.al (2003) and Whyte et.al, (2010) further suggested that both THC and CBD could modulate mitochondrial function and inhibit respiration, resulting in cell death.

Reports from Blithikioti et al., (2019) suggests that consumption of THC led to a decrease in psychomotor skills, which did not correlate with any altered brain activation. Regarding residual effects, King et al., (2011) found decreased psychomotor speed in chronic users performing a finger-tapping task but no corresponding cerebellar alteration. On the other hand, Lopez-Larson et al (2012) showed that a sample of adolescent heavy users performing a finger- tapping task had decreased cerebellar activation that was negatively correlated with lifetime exposure to marijuana, suggesting that the cerebellum might be particularly sensitive to cannabis effects during development and, therefore, associated with long-term alterations (King et al., 2011).

Several studies have suggested altered cerebellar function in chronic cannabis users (Daum et al., 1993; Safo and Regehr, 2005; Marcaggi 2015 & Koziol et al., 2014). Emerging evidence suggests that in addition to motor functions, the normal cerebellum plays a significant role in cognition in the healthy human brain (Koziol et al., 2014). It has an active role in a variety of mental activities, including facial recognition, emotion attribution, theory of mind attributions, directed attention, and many types of memory (Schmahmann, 1998). In functional imaging studies, cerebellar activations occur even when motor components of the tasks are well controlled. It is now widely accepted that many normal cognitive functions are performed by using distributed circuits that include cerebellar and thalamic components, with cortical components that vary depending on the nature of a given mental activity. Amaza et. al., (2013) in their work observed physical changes which include hyperactivity, increase in appetite as well as increase in weight. This sis due to the fact that endocannabinoids in the hypothalamus activate cannabinoids receptors that are responsible for maintaining food intake and also cannabis sativa has acute appetite enhancing effects, thereby increasing body weight in experiment model. This is consistent with the findings of this study.

From the result of this study, we can deduce that electron microscopy revealed greater details as to the nature of harm caused by *cannabis sativa* consumption on cerebellar neurons compared to light microscopy. Further aspects of this investigation are required on other parts of the brain to ascertain the extent of potential harm caused on the brain by cannabis sativa consumption.

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REFERENCES

Aartsma-Rus, A. and van Putten, M., 2014. Assessing functional performance in the mdx mouse model. JoVE (Journal of Visualized Experiments), (85), p.e51303.

Adebiyi, O.E., Olopade, F.E., Olopade, J.O. and Olayemi, F.O., 2016. Behavioural studies on the ethanol leaf extract of Grewia carpinifolia in Wistar rats. African health sciences, 16(1), pp.339-346.

Aguwa, U.S. and Onyejike, D.N., 2022. Neurobehavioral Effects of Anterior, Superior and Lateral Head Injury on Adult Male Wistar Rats. Asian Journal of Research and Reports in Neurology, 5(2), pp.17-26.

Almeida, R.G. and Lyons, D.A., 2017. On myelinated axon plasticity and neuronal circuit formation and function. Journal of Neuroscience, 37(42), pp.10023-10034.

Ashton, J.C., Dowie, M.J. and Glass, M., 2017. The endocannabinoid system and human brain functions: insight from memory, motor, and mood pathologies. In The Endocannabinoid System (pp. 115-186). Academic Press.

Authier, N., Gillet, J.P., Fialip, J., Eschalier, A. and Coudore, F., 2003. An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. Experimental neurology, 182(1), pp.12-20.

Battistella G, Fornari E, Annoni JM, Chtioui H, Dao K, Fabritius M, Favrat B, Mall JF, Maeder P, Giroud C (2014). Long-Term Effects of Cannabis on Brain Structure. Neuropsychopharmacology; 39:2041–2048.

Bahji, A., Stephenson, C., Tyo, R., Hawken, E. and Seitz, D., 2021. Prevalence of cannabis withdrawal symptoms among people with regular or dependent use of cannabinoids: A systematic review and meta-analysis. Biological Psychiatry, 89(9), p.S222.

Blithikioti, C., Miquel, L., Batalla, A., Rubio, B., Maffei, G., Herreros, I., Gual, A., Verschure, P. and Balcells-Oliveró, M., 2019. Cerebellar alterations in cannabis users: A systematic review. Addiction biology, 24(6), pp.1121-1137.

Bloomfield, M.A., Hindocha, C., Green, S.F., Wall, M.B., Lees, R., Petrilli, K., Costello, H., Ogunbiyi, M.O., Bossong, M.G. and Freeman, T.P., 2019. The neuropsychopharmacology of cannabis: A review of human imaging studies. Pharmacology & therapeutics, 195, pp.132-161.

Carleton, H.M., Drury, R.A.B. and Wallington, E.A., 1967. Histological technique (Vol. 3). London:: Oxford University Press.

Cassano, T., Villani, R., Pace, L., Carbone, A., Bukke, V.N., Orkisz, S., Avolio, C. and Serviddio, G., 2020. From Cannabis sativa to cannabidiol: Promising therapeutic candidate for the treatment of neurodegenerative diseases. Frontiers in pharmacology, 11, p.124.

Chang L, Cloak C, Yakupov R, Ernst T (2006). Combined and Independent Effects of Chronic Marijuana Use and HIV on Brain Metabolites. Journal of Neuroimmune Pharmacology; 1:65– 76

Chye, Y., Christensen, E. and Yücel, M., 2020. Cannabis use in adolescence: a review of neuroimaging findings. Journal of dual diagnosis, 16(1), pp.83-105.

Connor, J.P., Stjepanović, D., Le Foll, B., Hoch, E., Budney, A.J. and Hall, W.D., 2021. Cannabis use and cannabis use disorder. Nature Reviews Disease Primers, 7(1), p.16.

Daum, I., Schugens, M.M., Ackermann, H., Lutzenberger, W., Dichgans, J. and Birbaumer, N., 1993. Classical conditioning after cerebellar lesions in humans. Behavioral neuroscience, 107(5), p.748.

de Faria Jr, O., Pivonkova, H., Varga, B., Timmler, S., Evans, K.A. and Káradóttir, R.T., 2021. Periods of synchronized myelin changes shape brain function and plasticity. Nature Neuroscience, 24(11), pp.1508-1521.

Freels, T.G., Baxter-Potter, L.N., Lugo, J.M., Glodosky, N.C., Wright, H.R., Baglot, S.L., Petrie, G.N., Yu, Z., Clowers, B.H., Cuttler, C. and Fuchs, R.A., 2020. Vaporized cannabis extracts have reinforcing properties and support conditioned drug-seeking behavior in rats. Journal of Neuroscience, 40(9), pp.1897-1908.

Figueiredo, P.R., Tolomeo, S., Steele, J.D. and Baldacchino, A., 2020. Neurocognitive consequences of chronic cannabis use: a systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews, 108, pp.358-369.

Filley, C.M. and Fields, R.D., 2016. White matter and cognition: making the connection. Journal of neurophysiology, 116(5), pp.2093-2104.

Ford, T.C., Hayley, A.C., Downey, L.A. and Parrott, A.C., 2017. Cannabis: an overview of its adverse acute and chronic effects and its implications. Current drug abuse reviews, 10(1), pp.6- 18.

Goodhew, P., 2011. General introduction to transmission electron microscopy (TEM). Aberration-Corrected Analytical Transmission Electron Microscopy, pp.1-19.

Gordon, A.J., Conley, J.W. and Gordon, J.M., 2013. Medical consequences of marijuana use: a review of current literature. Current psychiatry reports, 15, pp.1-11.

Grant, I., Gonzalez, R., Carey, C.L., Natarajan, L. and Wolfson, T., 2003. Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. Journal of the International Neuropsychological Society, 9(5), pp.679-689.

Hall, W., Leung, J. and Lynskey, M., 2020. The effects of cannabis use on the development of adolescents and young adults. Annual Review of Developmental Psychology, 2(1), pp.461-483.

Harclerode, J., 1980. THE EFFECT OF MARIJUANA ON THE MALE. Marijuana Research Findings, 1980, 31, p.137.

Hasin, D.S., 2018. US epidemiology of cannabis use and associated problems. Neuropsychopharmacology, 43(1), pp.195-212.

Hindocha, C., Freeman, T.P., Schafer, G., Gardner, C., Bloomfield, M.A., Bramon, E., Morgan, C.J. and Curran, H.V., 2020. Acute effects of cannabinoids on addiction endophenotypes are moderated by genes encoding the CB1 receptor and FAAH enzyme. Addiction Biology, 25(3), p.e12762.

Iversen, L., 2005. Long-term effects of exposure to cannabis. Current Opinion in Pharmacology, 5(1), pp.69-72.

Jackson, N.J., Isen, J.D., Khoddam, R., Irons, D., Tuvblad, C., Iacono, W.G., McGue, M., Raine, A. and Baker, L.A., 2016. Impact of adolescent marijuana use on intelligence: Results from two longitudinal twin studies. Proceedings of the National Academy of Sciences, 113(5), pp.E500-E508.

Jouanjus, E., Raymond, V., Lapeyre-Mestre, M. and Wolff, V., 2017. What is the current knowledge about the cardiovascular risk for users of cannabis-based products? A systematic review. Current atherosclerosis reports, 19, pp.1-15.

King, G.R., Ernst, T., Deng, W., Stenger, A., Gonzales, R.M.K., Nakama, H. and Chang, L., 2011. Effects of chronic active cannabis use on visuomotor integration, in relation to brain activation and cortisol levels. The Journal of Neuroscience, 31(49), p.17923.

Klein, S.M., Vykoukal, J., Lechler, P., Zeitler, K., Gehmert, S., Schreml, S., Alt, E., Bogdahn, U. and Prantl, L., 2012. Noninvasive in vivo assessment of muscle impairment in the mdx mouse model–a comparison of two common wire hanging methods with two different results. Journal of Neuroscience Methods, 203(2), pp.292-297.

Koziol, L.F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., Ito, M., Manto, M., Marvel, C., Parker, K. and Pezzulo, G., 2014. Consensus paper: the cerebellum's role in movement and cognition. The Cerebellum, 13, pp.151-177.

Krist, A.H., Davidson, K.W., Mangione, C.M., Barry, M.J., Cabana, M., Caughey, A.B., Curry, S.J., Donahue, K., Doubeni, C.A., Epling, J.W. and Kubik, M., 2020. Screening for unhealthy drug use: US Preventive Services Task Force recommendation statement. Jama, 323(22), pp.2301-2309.

Lorenzetti, V., Chye, Y., Silva, P., Solowij, N. and Roberts, C.A., 2019. Does regular cannabis use affect neuroanatomy? An updated systematic review and meta-analysis of structural neuroimaging studies. European archives of psychiatry and clinical neuroscience, 269, pp.59-71.

Lorenzetti, V., Lubman, D.I., Whittle, S., Solowij, N. and Yücel, M., 2010. Structural MRI findings in long-term cannabis users: what do we know?. Substance use & misuse, 45(11), pp.1787-1808.

Linnaeus, C., Carl Linnaeus. Children, 1000, p.1739.

Lopez-Larson, M.P., Rogowska, J., Bogorodzki, P., Bueler, C.E., McGlade, E.C. and Yurgelun Todd, D.A., 2012.

Cortico-cerebellar abnormalities in adolescents with heavy marijuana use. Psychiatry Research: Neuroimaging, 202(3), pp.224-232.

Lucas, C.J., Galettis, P. and Schneider, J., 2018. The pharmacokinetics and the pharmacodynamics of cannabinoids. British Journal of Clinical Pharmacology, 84(11), pp.2477-2482.

Malabadi, R.B., Kolkar, K.P. and Chalannavar, R.K., 2023. Medical Cannabis sativa (Marijuana or Drug type); The story of the discovery of Δ9-Tetrahydrocannabinol (THC). International Journal of Innovation Scientific Research and Review, 5(3), pp.4134-4143.

Marcaggi, P., 2015. Cerebellar endocannabinoids: retrograde signaling from purkinje cells. The Cerebellum, 14, pp.341-353.

Mechoulam, R., 2012, February. Cannabis—a valuable drug that deserves better treatment. In Mayo Clinic Proceedings (Vol. 87, No. 2, pp. 107-109). Elsevier.

Oswald, I.W., Ojeda, M.A., Pobanz, R.J., Koby, K.A., Buchanan, A.J., Del Rosso, J., Guzman,

M.A. and Martin, T.J., 2021. Identification of a new family of prenylated volatile sulfur compounds in cannabis revealed by comprehensive two-dimensional gas chromatography. ACS omega, 6(47), pp.31667-31676.

Pajevic, S., Basser, P.J. and Fields, R.D., 2014. Role of myelin plasticity in oscillations and synchrony of neuronal activity. Neuroscience, 276, pp.135-147.

Poggi, G., Boretius, S., Möbius, W., Moschny, N., Baudewig, J., Ruhwedel, T., Hassouna, I., Wieser, G.L., Werner, H.B., Goebbels, S. and Nave, K.A., 2016. Cortical network dysfunction is caused by a subtle defect of myelination. Glia, 64(11), pp.2025-2040.

Pope, C., Mechoulam, R. and Parsons, L., 2010. Endocannabinoid signaling in neurotoxicity and neuroprotection. Neurotoxicology, 31(5), pp.562-571.

Sarafian, T.A., Kouyoumjian, S., Khoshaghideh, F., Tashkin, D.P. and Roth, M.D., 2003. Δ9- Tetrahydrocannabinol disrupts mitochondrial function and cell energetics. American Journal of Physiology-Lung Cellular and Molecular Physiology, 284(2), pp. L298-L306.

Safo, P.K. and Regehr, W.G., 2005. Endocannabinoids control the induction of cerebellar LTD. Neuron, 48(4), pp.647-659.

Schmahmann, J.D., 1998. Dysmetria of thought: clinical consequences of cerebellar dysfunction on cognition and affect. Trends in cognitive sciences, 2(9), pp.362-371.

Smith, A., Kaufman, F., Sandy, M.S. and Cardenas, A., 2020. Cannabis exposure during critical windows of development: epigenetic and molecular pathways implicated in neuropsychiatric disease. Current environmental health reports, 7, pp.325-342.

Whan, L.B., West, M.C., McClure, N. and Lewis, S.E., 2006. Effects of delta-9- tetrahydrocannabinol, the primary psychoactive cannabinoid in marijuana, on human sperm function in vitro. Fertility and sterility, 85(3), pp.653-660.

Whyte, D.A., Al-Hammadi, S., Balhaj, G., Brown, O.M., Penefsky, H.S. and Souid, A.K., 2010. Cannabinoids inhibit the cellular respiration of human oral cancer cells. Pharmacology, 85(6), pp.328-335

Yadima, S.G., Muhammad, M. and Usman, B., 2017. Phytochemical screening and antibacterial activities of aqueous extracts of Diospyros mespiliformis, Mitragyna inermis, Piliostigma reticulatum and Tamarindus indica in Northern Nigeria. Arid Zone J. Engin, Technol. & Environ, 13(3), pp.411-419.