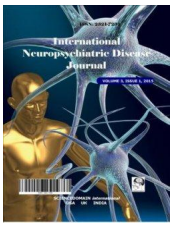

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Histomorphological Aberrations Associated with Cannabis and Caffeine Exposure in the Hippocampus of Juvenile Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JOO and OO designed the study, performed the animals treatment, wrote the protocol and wrote the first draft of the manuscript. Authors AJO and SYO managed the analyses of the study. All authors managed the literature searches and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Cannabis and caffeine are two psychoactive substances often abused. Cannabis is illegal in many countries, yet it constitutes a social menace as individuals still abuse the substance. Caffeine on the other hand is legal and used almost without restrictions in most countries. It is also important to note that adolescence is a critical period of neural and mental development; and the juvenile brain might be typically vulnerable to certain consequences of psychoactive agents use and abuse. Notably, most existing literatures have considered the effects of these substances on the adult and matured brain. The aim of this investigation, therefore, was to assess the effects of caffeine and cannabis use on the histomorphology of the hippocampus of juvenile Wistar rats. Seventy two Wistar rats of both sexes were divided into six groups named A-F. The Group A served as the Control. Group B were administered the higher dose of caffeine; Group C were administered the lower dosage of caffeine; Group D were administered the higher dosage of cannabis; Group E were administered the

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lower dosage of cannabis; Group F were administered caffeine and cannabis combined. Administration was done using suitable oral gavages and the animals were fed *ad libitum*. Animals were sacrificed by cervical dislocation after 21 days. The brain tissues were excised; fixed in formal saline and processed using the haematoxylin and eosin staining technique. Evaluation of the histoarchitecture of the hippocampal formation showed that caffeine and cannabis did not produce extensive disruption of the hippocampal; formation and might not be termed deleterious. However, especially at the high doses, they altered individual neurons morphologies and the spatial distribution of the cells in the Cornu Ammonis and dentate gyrus.

Keywords: Hippocampus; caffeine cannabis; juvenile; wistar rat.

1. INTRODUCTION

Caffeine is a central nervous system stimulant of the methylxanthine class of psychoactive drugs. It is the world's most widely consumed psychoactive drug but unlike many other psychoactive substance it is legal an unregulated in nearly all parts of the world. Caffeine is a naturally occurring substance found in the leaves, seeds and fruits of at least 63 plant species worldwide and is part of a group of compounds known as methylxanthines. The most common sources of caffeine are coffee, cocoa beans, kola nuts and tea leaves [1-4]. Cannabis is a flowering plant with tetrahy dracannabinol [THC] as its principal constituent and is obtained by curing the flower. Cannabis is a popular recreational drug around the world. The psychoactive effects of cannabis are known to be biphasic in nature. Cannabis- preparations that are used as illicit drugs- has been labelled an illegal drug in most countries of the world including Nigeria. These drugs are being prepared in different forms and the daily consumptions and dosages of these drugs are on the increase especially among adolescents. There are however reports that suggested that cannabis active phytochemicals can significantly influence memory, and more likely, negatively [5].

Adolescence is a transitional stage of physical, psychological, developmental growth and it generally occurs during the period from puberty to adulthood. This stage is characterized by vital neoplastic modifications of the brain synapses and nature of networks as well as cytological changes that tends to determine the subsequent adult life mental integrity. The hippocampus is a major component of the human brain. Humans and other mammals have two hippocampi located on each cerebral hemisphere. The hippocampus has two structurally defined parts that include the cornu ammonis (CA1 to CA4) and the dentate gyrus [6,7]. The hippocampus plays important roles in memory consolidation from short to long term memory and spatial navigation. Because the hippocampus plays a significant role in memory formation, damage to it and related systems is central to the amnesic syndrome [8]. The hippocampus plays an important role in the formation of new memories and also experienced events. Other functions of the hippocampus' involvement include the detection of events, places and stimuli. Some researchers regard the hippocampus as part of the medial temporal lobe memory system responsible for general declarative memory [9,10].

Physical performance may be improved following caffeine ingestion [11], also, mental performance [12] and muscle endurance during brief intense exercise [13]. Caffeine enhances self-rated moods [11,14]. Although there have been reports of caffeine causing anxiety, a number of reviews of the research have shown that only high levels of caffeine bring on anxiety. Large amounts of caffeine late in the evening may interfere with the normal onset of sleep [12,15].

A well established effect of acute effects of cannabis in man is an impairment of short-term memory [16-18]. Many studies have shown significant effects on short-term memory, particularly when tests were used that depend heavily on attention. Animal studies have also found that THC, synthetic cannabinoids and anandamide cause deficits in short-term memory in spatial learning tasks [19-21]. These include delayed matching or non-matching tests in rodents [22,20], performance in a radial arm maze [23] and a fixed ratio food acquisition task in squirrel monkeys.

The effects of both cannabinoids [23] and anandamide [22] were reversed by rimonabant, indicating that they are mediated by the cannabinoid receptor type 1 [CB1] receptor [19-21,24]. Reports claimed that the effects of the

treatment of rats with cannabinoids on short-term memory in a delayed non-matching to sample test were equivalent to the effects seen after surgical removal of the hippocampus. In either case, animals were unable to segregate information between trials in the task because of disruptions to the processing of sensory information in hippocampal circuits. CB1 receptors are expressed at high densities in the hippocampus. They are particularly abundant on the terminals of a sub-set of GABAergic basket cell interneurons, which also contain the neuropeptide cholecystokinin [25], and this is also the case in human hippocampus [26,27]. These are presumably the GABAergic neurons involved in the endocannabinoid-mediated [depolarisation-induced suppression of inhibition] DSI phenomenon. The terminals of these cells surround large pyramidal neuron somata in the CA1- CA4 fields. GABAergic neurons in the dentate gyrus also express CB1 receptors, with terminals concentrated at the boundary of the molecular and granule cell layers [28]. In addition CB1 receptors are expressed, at a lower level, in the glutamatergic pyramidal cells and their terminals. Cannabinoids can thus inhibit both the release of GABA and glutamate in hippocampal circuits.

It is however interesting to note that not many investigations have considered the direct effects of caffeine and cannabis phytochemicals [THC and cannabinoid] on the ultrastructure of the hippocampus in order to appreciate the structural causes of the functional aberrations that are typically associated with their uses. The availability of such knowledge will also help further quest into addressing these challenges using remedial approaches or to prevent them. To this end, this investigation was carried out to study the effects of caffeine and cannabis sativa on the hippocampal structure using histological procedures.

The aim of this investigation was to observe the nature of effects produced by caffeine and cannabis on the hippocampus of adolescent rats, with emphasis on its histoarchitecture.

2. MATERIALS AND METHODS

A total of 72 adolescent rats of both sexes were used for the experiment. The rats were placed in six groups, each group containing 12 rats. The rats were 5 weeks old, weighing approximately 100 g. The groups were labelled as follows:

Group A: Group A served as the control group; rats in this group were only given feed and water.

Group B: Group B animals were administered the high does caffeine to observe caffeine effects at relatively high dose of ingestion. Rats in this group were administered 100 mg/kg body weight of caffeine, daily.

Group C: Group C animals were administered the lower caffeine dose to observe caffeine effects at relatively lower dose of ingestion. Rats in this group were administered 50 mg/kg body weight of caffeine, daily.

Group D: Group D animals were administered the high dose of *Cannabis sativa* to observe the cannabis effects at relatively high dose. Rats in this group were administered 500 mg/kg body weight of cannabis, daily.

Group E: Group E animals were administered the relatively low dose of *Cannabis sativa* to observe the cannabis effects at relatively low dose. Rats in this group were administered 200 mg/kg body weight of cannabis, daily.

Group F: Group F animals were administered a combination of relatively low doses of caffeine and *Cannabis sativa*. Rats in this group were administered 200 mg/kg body weight of cannabis plus 50 mg/kg body weight caffeine, daily.

Aqueous cannabis extract was prepared from dried cannabis and suitably mixed with water to obtain desired doses as designed in the experimental regimens. Air-dried cannabis was grounded into fine powder, soaked in distilled water for twenty-four hours and thereafter filtered. Extract was evaporated to dryness at moderated temperature. Anhydrous caffeine powder was dissolved in distilled water to also obtain the standard doses. The experiment took place in the standard animal holding facility and the rats were housed, handled bred and treated with adherence to ethical and standard research practices; according to the guide to the care and use of animals in research and teaching protocol. The animals were first allowed to acclimatise. The rats were fed with pelleted feed and water during this period; their beddings were also changed regularly and good hygiene was maintained. The administration of the substances lasted 21 days. Experimental animals were sacrificed by cervical dislocation, and excision of tissue was done by dissecting the skull. Histological processing was carried out using the

H&E [29] and the Luxol Fast Blue [30] techniques. Photomicrographs of the tissues were obtained using the Accuscope Photomicrographic Set. Representative photomicrographs are presented in figures and analysed following standard histomorphological principles [31].

3. RESULTS

Photomicrographs show structural changes in treatment groups compared to control group in dentate gyrus, cornu ammonis (CA4) regions of the hippocampus. The aberrations could be associated with the effects of the administered substances, considering their chemical nature and the doses administered. The high caffeine dose affected the dentate gyrus architecture the more (Fig. 1) while the high cannabis does affected the pyramidal cells the more (Fig. 2). The combination of both showed effects on both areas.

4. DISCUSSION

The basic features of the hippocampal formation and are well demonstrated in the control group. The dentate gyrus and its granular cells as well as the pyramidal cells including the Cornu Ammonis were clearly defined in the control group.

When the high caffeine dose was employed the hippocampal formation was still largely preserved. The spatial distribution of the Cornu Ammonis cells were however disrupted with the cells being farther apart. Also, the cells- granular are morphologically heterogeneous relative to the control. The animals that were administered caffeine low dose had all their basic features of the hippocampus preserved and defined. Cells are mildly heterogeneous however. Also, the Cornu Ammonis is relatively less intact. This observation suggests that caffeine at this dosage altered cell spatial disruption mildly; it however did not cause extensive disruption to the hippocampus. There is therefore evidence that caffeine use altered hippocampal ultrastructure over time, and the gravity of effects increased with dosage. This might explain in part why caffeine could later certain behavioural and cognitive attributes. It also suggests that such effects as observed in behaviour and cognition have structural basis. Though not many research have considered such structural changes; it has been documented that caffeine has biochemical effects in the brain and throughout the body that could influence neurogenesis. While caffeine has been reported to improve short term mental and physical performances [31]; prolonged use has been suggested to inhibit hippocampal neurogenesis [32]. Yet a number of literatures support its memory enhancement potentials [33-36].

The use of high dose cannabis produced certain observable effects- pyramidal cells are mildly heterogeneous, thought there were still prominently demonstrated. Cornu Ammonis cells are preserved and the cells are intact in terms of spatial placement relative to the control. The adjacent white matter region however shows signs of slight disruption. Thus, cannabis at the high dose had observable effects on pyramidal cell morphologies and the adjacent white matter in some regions. The administration of the low dose of cannabis appears not to cause any major disruption or damage to the hippocampal formation. Interestingly the dentate gyrus cells are quite prominently demonstrated in this group, and relatively abundant. The pyramidal cells are also prominently demonstrated. Though cannabis has been reported to disrupt short term memory; it appears not to alter the hippocampal structure extensively at the low dosage. Cannabis on the other hand has been suggested to have potential to promote hippocampal neurogenesis [37]. Yet, cannabis uses are being associated with brain structural aberrations [38,39].

The combination of cannabis and caffeine in the Group F also produced observable effects; but did not produce observable extensive damage to the hippocampal formation. The granular cells of the dentate gyrus are less compact in their relative distribution. Also, the pyramidal cells are slightly heterogeneous. The observation suggests that the combination of caffeine and cannabis will alter the hippocampal cells morphologies when combined even at lower doses.

The above mentioned observations could provide insight into the structural changes that accompany the cognition and behavioural changes that have been reportedly associated with the use of caffeine [29] and cannabis. It is however not enough to establish whether these changes could be reversible, considering the possibility of the hippocampal adult neurogenesis. The structural and cellular aberrations as observed might alter memory consolidation mechanism especially if they interfered with adult neurogenesis which is linked to memory formation [40,41].

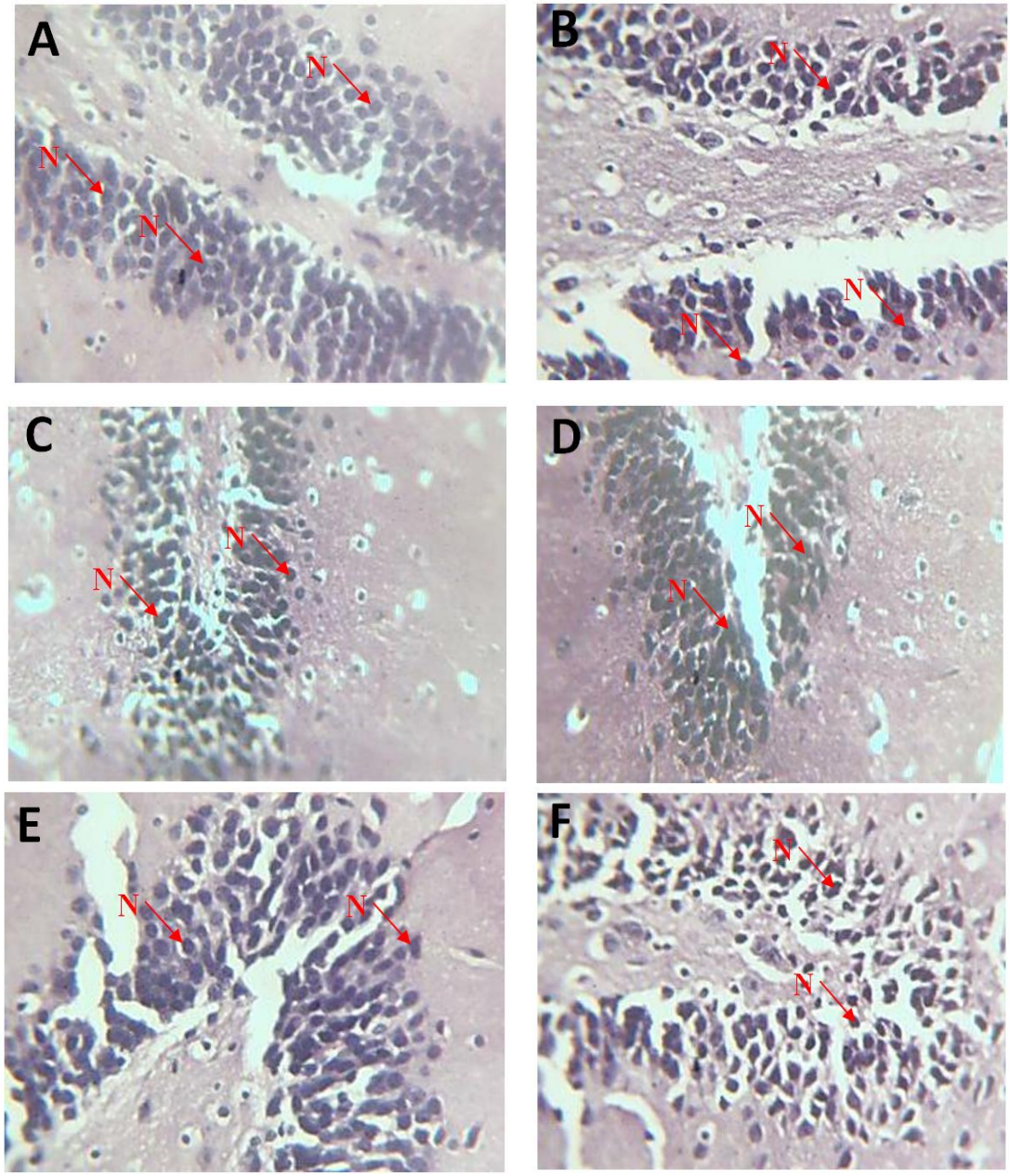

Fig. 1. Photomicrographs of the dentate gyrus of the Groups A-F animals that were used for the experiment. The administered agents- caffeine and cannabis, did not produce extensive structural disruption of the dentate gyrus. The combination of cannabis and caffeine [Group F] however caused observable sparsely populated dentate gyrus relative to the control [Group A]. [N=Neuron; X640]
A-control, B-high does caffeine [100 mg/kg], C-Lower caffeine [50 mg/kg], C-high dose of *Cannabis* [500 mg/kg], E - low dose of *Cannabis* [200 mg/kg], F-combination caffeine + *Cannabis* [200 mg/kg +50 mg/kg]

The Luxol fast blue staining technique demonstrates the myelin sheet integrity within the hippocampal formation. The fibre demonstration across the group showed that administered agents in their various doses and combination did not cause

observable or extensive damage to the fibre bundles. This again supports the morphological observation that there was no extensive disruption of the hippocampal formation but only localized tissue damage in the stated tissue damage.

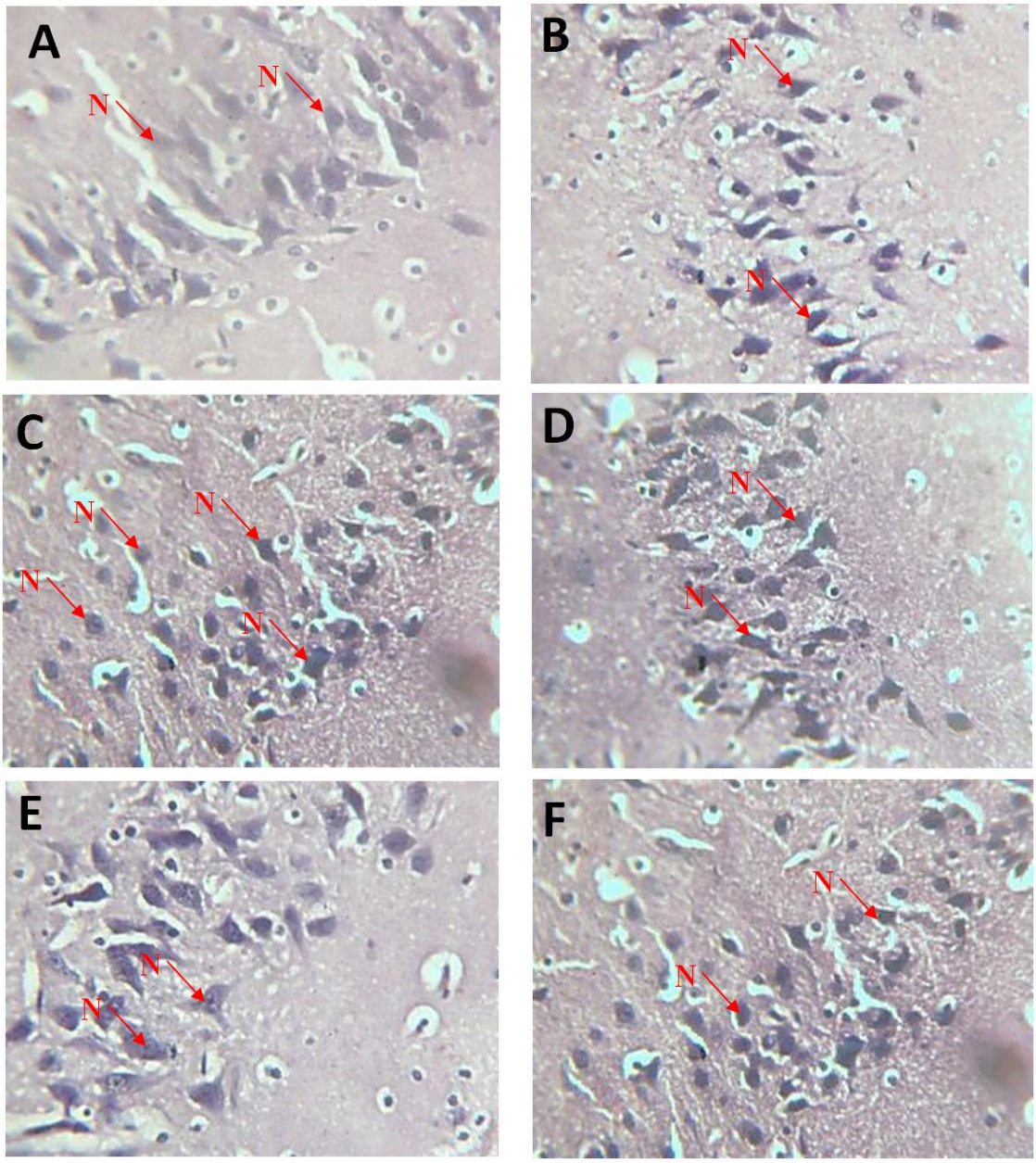

Fig. 2. Photomicrographs of the hippocampal Cornu Ammonis [CA 4] of the Group A-F animals that were used for the experiment. There are no signs of extensive disruptions of the CA. High doses of cannabis [Group D] and the combination of cannabis and caffeine [Group E] caused mild cellular heterogeneity in this groups. [N=Neuron; X640]
A-control, B-high does caffeine [100 mg/kg], C-Lower caffeine [50 mg/kg], C-high dose of *Cannabis* [500 mg/kg], E - low dose of *Cannabis* [200 mg/kg], F-combination caffeine + *Cannabis* [200 mg/kg +50 mg/kg]

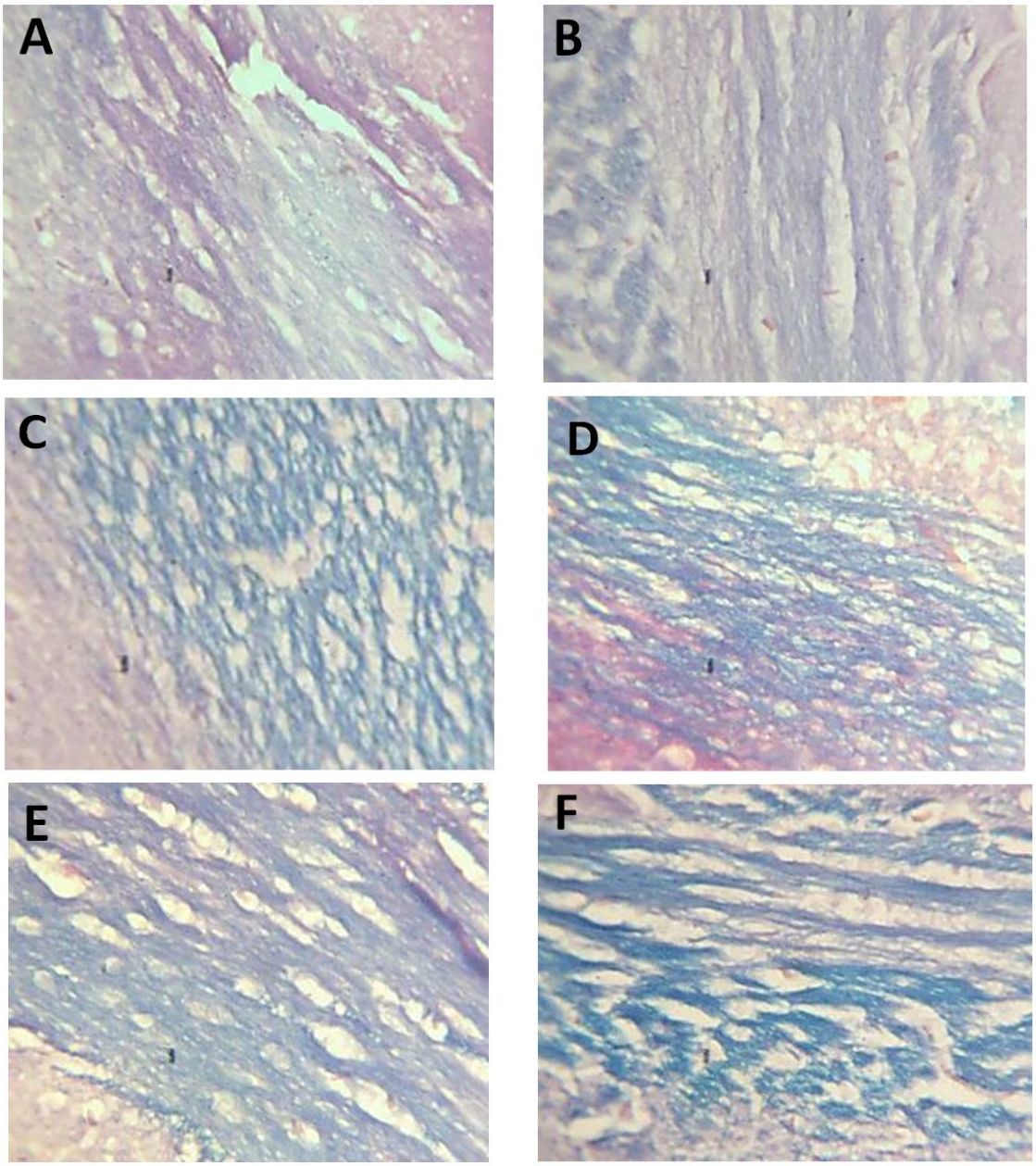

Fig. 3. Photomicrographs of the brain tissue, demonstrating hippocampal fibre myelin sheath integrity in Groups A-F using the Luxol Fast Blue staining technique. There are no observable disruptions or extensive damage to the fibre bundles. [X640]
A-control, B-high does caffeine [100 mg/kg], C-Lower caffeine [50 mg/kg], C-high dose of *Cannabis* [500 mg/kg], E - low dose of *Cannabis* [200 mg/kg], F-combination caffeine + *Cannabis* [200 mg/kg +50 mg/kg]

5. CONCLUSION AND RECOMMENDATION

Caffeine and cannabis extract as used did not produce extensive disruptions of the hippocampal formation. They can therefore not be described as being deleterious. However, they possess the potential, especially at the high doses to later the spatial distribution of cell and the morphologies of the individual cells. These

observations might point to their roles in including memories especially, their mechanism of consolidation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol. 1996; 34(I):119-29.
2. Muñiz JA, Gomez G, González B, Muñiz JA, Gomez G, González B, Rivero-Echeto MC, Cadet JL, García-Rill E, Urbano FJ, Bisagno V. Combined effects of simultaneous exposure to caffeine and cocaine in the mouse striatum. Neurotoxicity Research. 2016;29:525.
3. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacological Reviews. 1999;51:83-133.
4. Nehlig A. Are we dependent upon coffee and caffeine? A review on human and animal data. Neuroscience & Biobehavioral Reviews. 1999;23:563–576.
5. Hájos N, Katona I, Naiem SS. Cannabinoids inhibit hippocampal GAB AERGIC transmission and network oscillations. Eur J Neurosci 2000;12: 3239–3249.
6. O'Keefe J, Nadel L. The hippocampus as a cognitive map. Oxford University Press. Elphick MR, Egertova ÂM. The neurobiology and evolution of cannabinoid signalling. [Review]. Philos Trans R Soc Lond B Biol Sci. 2001;356:381-408.
7. Amaral D, Lavenex P. Chpter 3: Hippocampal neuroanatomy. In Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J. The hippocampus book. Oxford University Press; 2006.

   ISBN: 978-0-19-510027-3
8. Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery and Psychiatry. 1957;20(1): 11–21.

   DOI: 10.1136/jnnp.20.1.1
9. Van Elzakker M, Fevurly RD, Breindel T, Spencer RL. Environmental novelty is associated with a selective increase in Fos expression in the output elements of the hippocampal formation and the perirhinal cortex. Learning & Memory. 2008;15(12): 899–908.

   DOI: 10.1101/lm.1196508

   PMC: 2632843

   PMID: 19050162
10. Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological Review. 1992;99(2):195–231.

    DOI: 10.1037/0033-295X.99.2.195

    PMID: 1594723
11. Magkos F, Kavouras SA. Caffeine and ephedrine: Physiological, metabolic and performance enhancing effects. Sports Med. 2004;34(13):871–889.
12. Smith PM, Doherty M. Effects of caffeine ingestionon rating of perceived exertion during and after exercise: A meta-analysis. Scandinavian Journal of Medicine and Science in Sport. 2005;15:69-78.
13. Jackman M, Wendling P, Friars D, Graham T. Metabolic catecholamine, and endurance responses to caffeine during intense exercise. Journal of Applied Physiology. 1996;81:1658-1663.
14. Lieberman HR. The effects of ginseng, ephedrine and caffeine on cognitive performance, mood and energy. Nutr Rev. 2001;59:91–102.
15. Bonnet M, Tancer M, Uhde T, Yeragani VK. Effects of caffeine on heart rate and QT variability during sleep. Depress Anxiety. 2005;22(3):150-5.
16. Jones RT. Marihuana: Human effects. In: Iversen LL, Iversen SD, Snyder SH, editors. Handbook of psycho-pharmacology. New York: Plenum Press. 1978;12:373–412.

17. Miller LL, Branconnier RJ. Cannabis: Effects on memory and the cholinergic limbic system. Psychol Bull. 1983;93:441-456.
18. Earleywine M. Understanding marijuana. Oxford: Oxford University Press; 2002.
19. Hampson AJ, Grimaldi M, Axelrod J. Cannabidiol and delta 9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci USA. 1998;95:8268-73.
20. Hampson RE, Deadwyler SA. Cannabinoids, hippocampal function and memory. Life Sci. 1999;65:715-23.
21. Hampson RE, Deadwyler SA. Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. J Neurosci. 2000;20: 8932-42.
22. Mallet PE, Beninger RJ. The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta 9-tetrahydrocannabinol or anandamide. Psychopharmacology (Berl). 1998;140:11-9.
23. Lichtman AH, Martin BR. Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology (Berl). 1996;126:125–31.
24. Deadwyler SA, Hampston RE, Childers SR. Functional significance of cannabinoid receptors in brain. In: Pertwee RG, editor. Cannabinoid Receptors. London: Academic Press. 1995;206-31.
25. Katona I, Sperlagh B, Sik A. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci. 1999;19:4544-58.
26. Katona I, Sperlagh B, MagloÂczky Z. GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience. 2000;100: 797-804.
27. Katona I, Rancz EA, AcsaÂdy L. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci. 2001;21:9506-18.
28. Egertova ÂM, Elphick MR. Localization of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB1. J Comp Neurol. 2000; 422:159-71.
29. Robert DC, Miller CH, Robert KJ. Cold sprng protocol herbs; 2014.
DOI: 10, 1101.
30. Sheehan D, Hrapchak B. Theory and practice of histotechnology, 2nd ed, Battelle Press, Ohio. 1980;262-264.
31. Garman RH. Histology of the central nervous system. Toxicol Pathol. 2011; 39(1):22-35.
32. Myoung-Eun H, Kyu-Hyun P, Sun-Yong B, Bong-Seon K, Jae-Bong K, Hak-Jin K, Sae-Ock O. Inhibitory effects of caffeine on hippocampal neurogenesis and function. Biochemical and Biophysical Research Communications. 2007;356:976–980.
33. Riedel WJ, Jolles J. Cognition enhancers in age-related cognitive decline. Drugs Aging. 1996;8:245–274.
34. Riedel W, Hogervorst E, Leboux R, Verhey F, van Praag H, Jolles J. Caffeine attenuates scopolamine-induced memory impairment in humans. Psycho-pharmacology (Berl). 1995;122:158–168.
35. Gevaerd MS, Takahashi RN, Silveira R, Cunha C. Caffeine reverses the memory disruption induced by intra-nigral MPTPinjection in rats. Brain Res. Bull. 2001;55:101–106.
36. Prediger RD, Pamplona FA, Fernandes D, Takahashi RN. Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD)—the spontaneously hypertensive rat (SHR). Int. J. Neuropsychopharmacol. 2005;8:583–594.
37. Wen J, Yun Z, Lan X, Jamie Van C, Shao-Ping J, Guang B, Xia Z. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. J Clin Invest. 2005;115(11):3104-3116.
38. Ashtari M, Avants B, Cyckowski L, Cervellione KL, Roofeh D, Cook P, Gee J, Sevy S, Kumra S. Medial temporal structures and memory functions in adolescents with heavy cannabis use. J Psychiatr Res. 2011; 45(8):1055-66.
39. Yücel M, Solowij N, Respondek C, Whittle S, Fornito A, Pantelis C, Lubman DI. Regional brain abnormalities associated

with long-term heavy cannabis use. Arch Gen Psychiatry. 2008;65(6): 694-701.

40. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memorie. Nature. 2001;410:372–376.

41. Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature. 1997;386:493–495.

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