



Caffeine and Cannabis Effects on the Cerebellar Cortex of Juvenile Rats

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Authors' contributions

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

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ABSTRACT

Caffeine and cannabis are two of the world most popularly consumed psychoactive substances. While caffeine is consumed with little or no regulation and restrictions in most of the countries of the world; cannabis safety of consumption has been queried severally and as such, it is labeled an illegal drug in many countries. It is important to appreciate what the effects of these two substances could be especially in juvenile models with an attempt to mimic the real life scenarios where several people consume these drugs as teenagers and adolescents. To this end, 72 juvenile Wistar rats were distributed into six groups labeled A-E. Group A served as the control and the animals were only fed *ad libitum*; Group B were administered the lower dosage of caffeine; Group C were administered the higher dosages of caffeine; Group D were administered the lower dosage of cannabis; Group E were administered the higher dosage of cannabis while the Group F were administered the both caffeine and cannabis- each substance being the lower dosage. The administration lasted 21 days and the animals were sacrificed thereafter. The cerebellum was excised in each animal; fixed in formal saline and then processed using the Haematoxyline and Eosin staining technique to observe the histological structures of the tissues. Results were taken in forms of photomicrographs, and analysed. Observations show that the higher doses of the agents used had effects that could be deleterious on the cerebellar architecture, especially by morphologically distorting the Purkinje cells.

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1. INTRODUCTION

The cerebellum is a part of the brain located posteriorly, and sits below the occipital lobe of the skull. Its primary function is motor coordination. It overlies the dorsal surfaces of the pons and medulla oblongata and contributes to the formation of the roof of the fourth ventricle [1]. The cerebellum is connected to the midbrain, pons, and medulla oblongata by three pairs of peduncles [1]. It may also be involved in some cognitive functions such as attention and language, and in regulating fear and pleasure responses [2]. The cerebellum does not initiate motor movement, but it contributes to coordination, precision, and accurate timing. It receives input from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine-tune motor activity [3]. Cerebellar damage produces disorders in fine movement, equilibrium, posture, and motor learning [3]. Specific functions of the cerebellum therefore include Maintenance of balance and posture [1]; coordination of voluntary movements [1]; motor learning [1]; and cognitive functions [3]. Two types of neuron play major roles in the cerebellar circuit: Purkinje cells and granule cells. Three types of axons also play dominant roles: mossy fibers and climbing fibers (which enter the cerebellum from outside), and parallel fibers (which are the axons of granule cells). There are two main pathways through the cerebellar circuit, originating from mossy fibers and climbing fibers, both eventually ending in the deep cerebellar nuclei [4]. Mossy fibers project directly to the deep nuclei, but also give rise to certain pathways. Climbing fibers project to Purkinje cells and also send collaterals directly to the deep nuclei [4]. The cerebellar cortex has three distinct cell layers: deep granular cells layer, the Purkinje cells layer and the outer molecular layer of stellate cells. Both stellate and basket cells form GABAergic synapses onto Purkinje cell dendrites [4]. Lesion to the cerebellum could result in Decomposition of movement, intention tremor, dysdiadochokinesia and deficits in motor learning [4].

A vital and very crucial stage of brain development is the adolescence stage [5]; in the experimental animals [Wistar rats], the postnatal days 28–49 correspond with human adolescent development [6]. This stage is associated with a lot of brain network modifications and cytological changes which will ultimately determine the

subsequent mental wholeness. Caffeine and cannabis are one of the world most consumed psychoactive drugs which are often abused by adolescents [7,8,9]. The endogenous cannabinoid system plays a key role in neurogenesis, neural specification, neural maturation, neuronal migration, axonal elongation, and glia formation [10]. Hence, adolescent cannabis use may permanently alter neuro-developmental trajectories, particularly in cannabinoid 1 receptor, CB1R-rich areas such as the cerebellum. Indeed [11]. A recent report stated that cannabis users exhibited a 23.9% decrease in cerebellar white matter as assessed with structural MRI, a decrease that was related to the duration of cannabis exposure [11]. In another recent study [12] demonstrated that 28-day abstinent adolescent cannabis users had increased cerebellar vermis volumes, which were associated with poorer executive function. The cerebellar vermal data were interpreted as suggesting that adolescent cannabis use disrupts normal cerebellar gray matter pruning processes during development [12].

Taken together, these studies suggest that early cannabis exposure can alter cerebellar structure, which may have lasting effects on synaptic plasticity and learning with the cerebellum. It is well known that the striatum is strongly involved in the regulation of motor behavior in animals, and presumably in humans, and the ability of caffeine to stimulate motor behavior is well documented and summarized [13,14,15]. Caffeine has been shown to alter neuronal activities and microglia morphology, but has not been established to cause extensive morphological alterations of pathological importance [16]

The aim of this investigation is to observe the nature of the effects produced independently and dependently by caffeine and cannabis on the cerebellum of the rat models and to compare possible variations in the effects of the two agents on relevant parameters.

2. MATERIALS AND METHODS

Seventy two adolescent albino rat models were used, comprising of both male and female evenly distributed from a breeding stock maintained in the animal holding facility of the Babcock University, Nigeria. Each of the rats were weighed and recorded. The rats were allowed to

acclimatize. The animals were divided into 6 groups, and were administered caffeine and cannabis according to the defined regimen as follows:

Group A: (control)- This was the control group which comprised of both male and female rat models, which had access to water and were fed with normal rat pellets.

Group B: (caffeine high dose)- This group was administered 100 mg/kg of caffeine via the oral gastric cannula. This group also comprised of both male and female and were fed also

Group C: (caffeine low dose)- This group was administered 50 mg/kg of caffeine via the oral gastric cannula. This group also comprised of both male and female and were fed also

Groups D: (cannabis high dose)- This group was administered 500 mg/kg of cannabis via the oral gastric cannula. This group also comprised of both male and female and were fed also

Group E: (cannabis low dose)- This group was administered 200 mg/kg of cannabis via the oral gastric cannula. This group also comprised of both male and female and were fed also

Group F: (cannabis low + caffeine low dose)- This group was administered 50 mg/kg of caffeine and 200 mg/kg of cannabis via the oral gastric cannula. This group also comprised of both male and female and were fed also

Regimen was determined by careful calculations from reported human use of the substances that were further subjected to a pilot study before the main experiment [7-16]. The administration lasted for 21 days; animals were sacrificed by cervical dislocation 24 hours after the last administration. Animals were dissected and the cerebellar tissues were excised and fixed in formal saline. Housing, handling and treatments of animals were done in compliance with institutional ethical and research standard practices. The tissues were taken through the routine tissue processing technique and sectioned with the rotary microtome at 50 μ thickness. Tissue sections were mounted on glass and stained using the Haematoxylin and Eosin staining [17]) and the Luxol Fast Blue [18] techniques to demonstrate the tissue

histoarchitecture and the myelination integrity respectively. Photomicrographs were obtained using the Accuscope Photomicrographic Set and structural analysis was done using fundamental qualitative histological principles [19] Photomicrographs of the H&E stained tissues across the groups were presented as follows: A demonstrates the cerebellum in its cross section showing the external granular layer, middle Purkinje cells and deep granular cells with the white matter at the core [X160]; B demonstrates the cellular elements of the cerebellar cortex and the neuropil [X640]; C demonstrates the cross section in relation to the core white matter [X640].

3. RESULTS AND DISCUSSION

3.1 Cerebellar Cortical Integrity

The cerebellar cortex of the group A animals (Control) is demonstrated (Fig. 1) showing a cross section of the cortex (Fig. 1A) as well as the cellular elements (Fig. 1B and C) in the cerebellar cortex. This cortex is normal and serves as a suitable standard reference for the other groups. Fig. 2 shows the cerebellar cortex of the Group B animals that were administered the low caffeine dose; the cerebellar cortex is normally demonstrated in this group and its molecular layer. Purkinje cells and the granule cells are clearly demonstrated. The observation showed that the cerebellar cortex is relatively normal in this group and the administered substance did not produce any deleterious effect. It is thus logical to infer that caffeine at this dosage is not deleterious to the cerebellar cells and did not alter their morphologies and spatial distribution. When the high dose of caffeine was administered to the animals (Group C); the cerebellar cortex as well as its cells are still largely preserved and there is no evidence of extensive tissue damage or destruction. This also implies that caffeine high dose as used did not cause cell death or observable morphological distortions or deformation. Hence, while caffeine is a psychoactive substance that influences neuronal activities; it does not at relatively high doses destroy cells or seriously deform them morphologically. Previous reports have not specifically addressed structural changes or effects caused by caffeine in the cerebellum. However, it reportedly has the potential to reorganise cortical synapses [20].

Photomicrographs

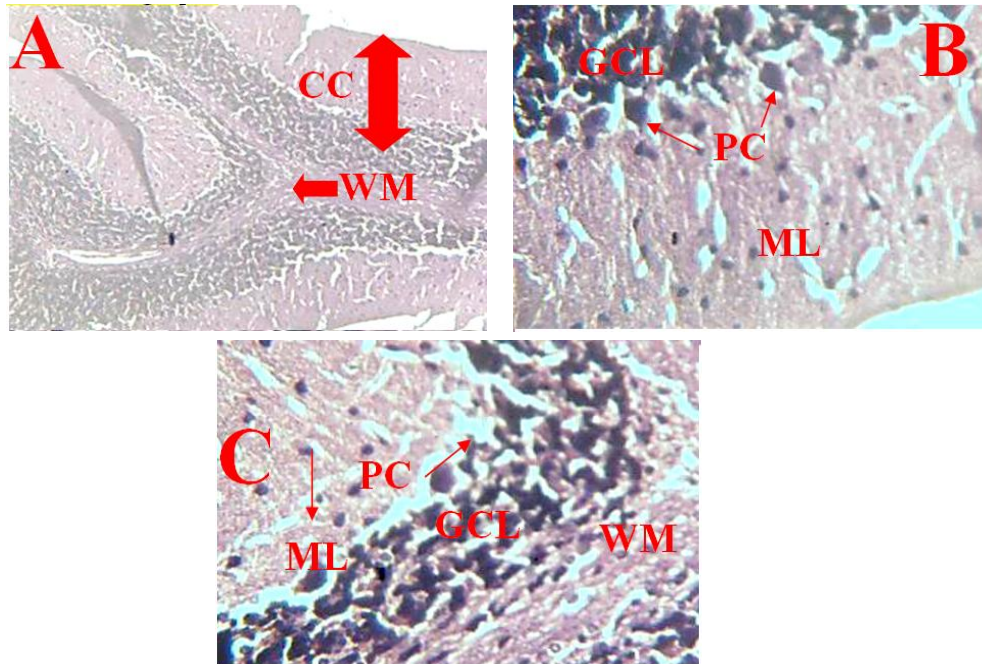


Fig. 1. Photomicrographs of the Control Group A animals, demonstrating the cerebellum using the Haematoxylin and Eosin staining technique. Cerebellar features are normal, serving as a suitable standard reference

CC= Cerebellar Cortex; WM= White Matter; ML= Molecular Layer; PC= Purkinje Cell; GCL= Granular Cell Layer

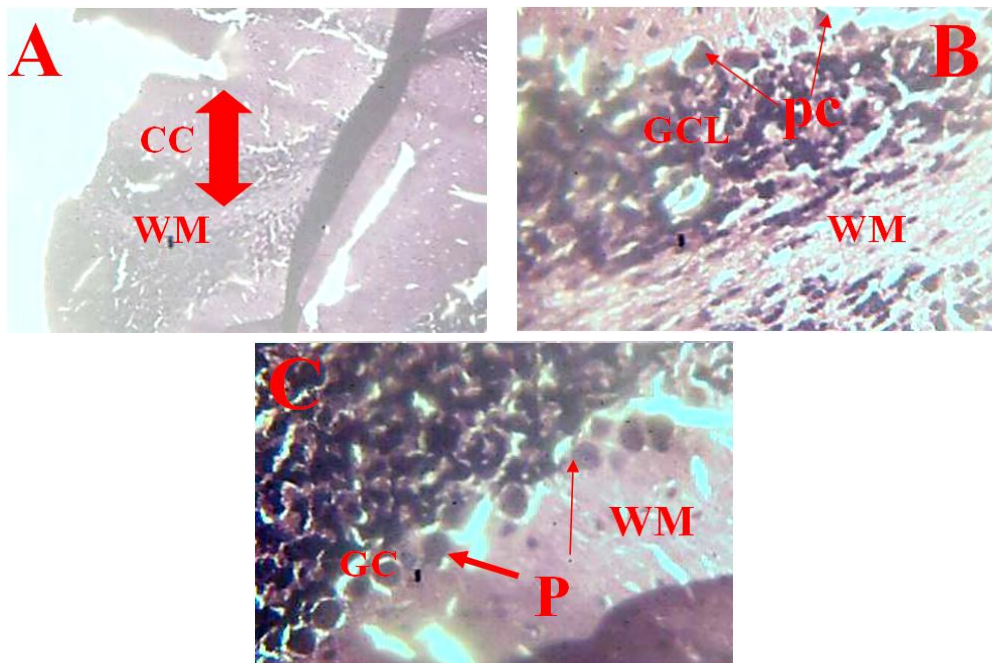


Fig. 2. Photomicrograph of the cerebellum of the Group B animals that were administered the caffeine low dose using haematoxylin and eosin staining technique. Cells are normally demonstrated and there are no signs of disruptions

CC= Cerebellar Cortex; WM= White Matter; ML= Molecular Layer; PC= Purkinje Cell; GCL= Granular Cell Layer

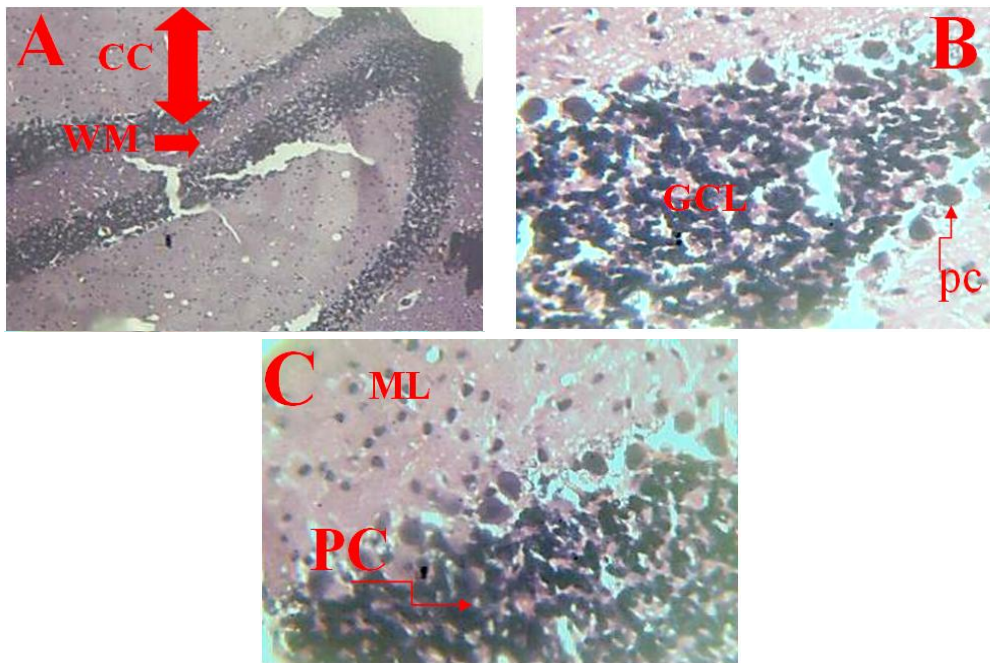


Fig. 3. Photomicrograph of the cerebellum of the Group C animals that were administered the caffeine high dose using haematoxylin and eosin staining technique. Cerebellar cortex histo- and cytoarchitecture are largely preserved.

CC= Cerebellar Cortex; WM= White Matter; ML= Molecular Layer; PC= Purkinje Cell; GCL= Granular Cell Layer

Cannabis High Dose

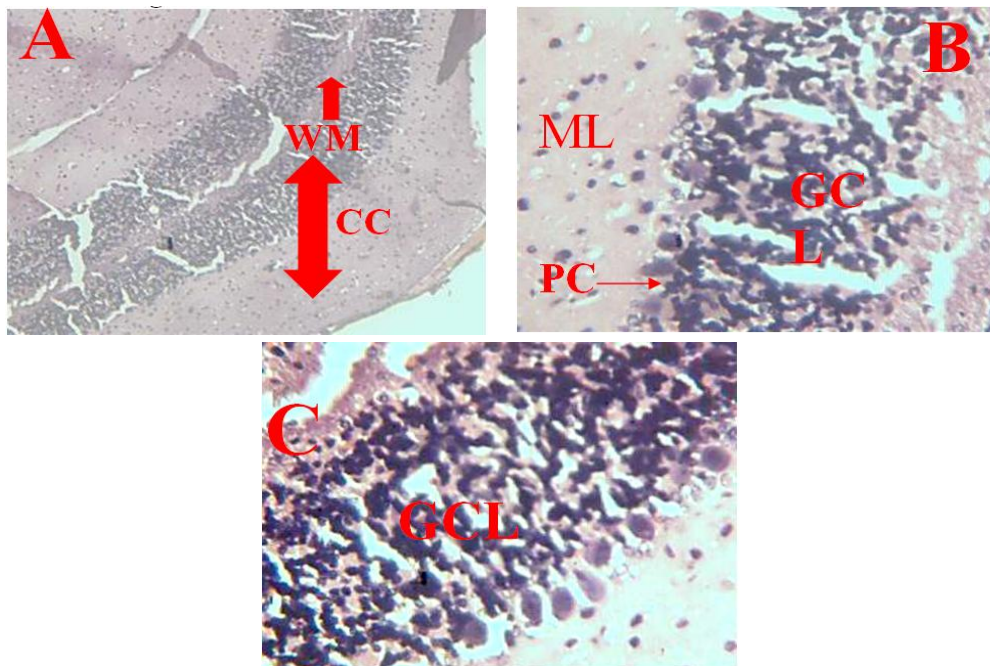


Fig. 4. Photomicrograph of the cerebellum of the Group E animals that were administered cannabis low dose using haematoxylin and eosin staining technique. Cortical tissue is largely preserved

CC= Cerebellar Cortex; WM= White Matter; ML= Molecular Layer; PC= Purkinje Cell; GCL= Granular Cell Layer

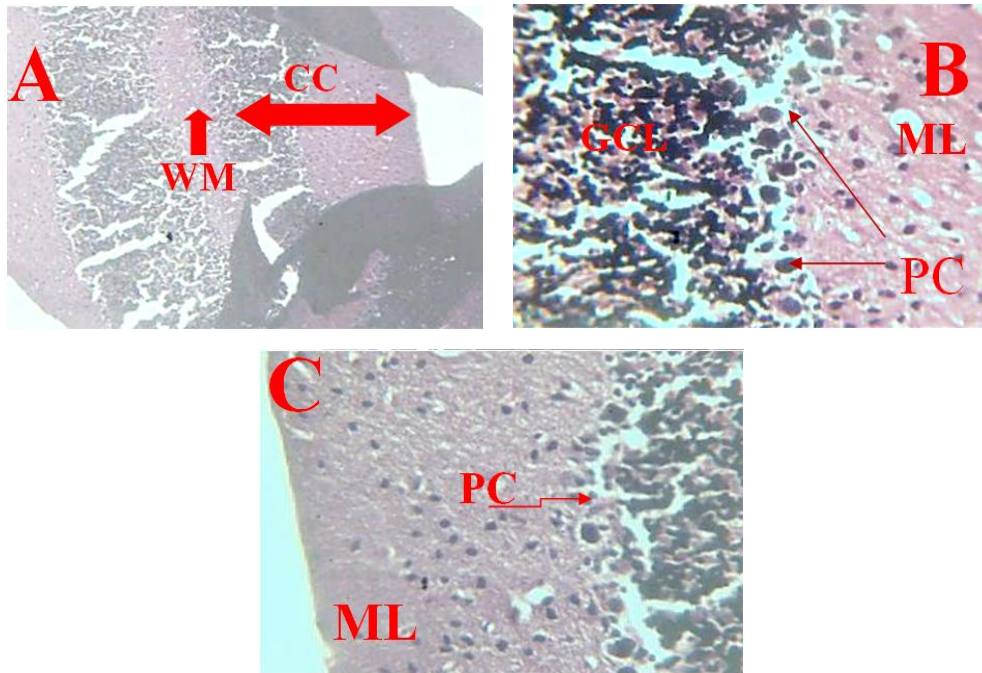


Fig. 5. Photomicrograph of the cerebellar cortex of the animals that were administered cannabis high dose using the haematoxylin and eosin staining technique. There are signs of Purkinje cells deformation
CC= Cerebellar Cortex; WM= White Matter; ML= Molecular Layer; PC= Purkinje Cell; GCL= Granular Cell Layer

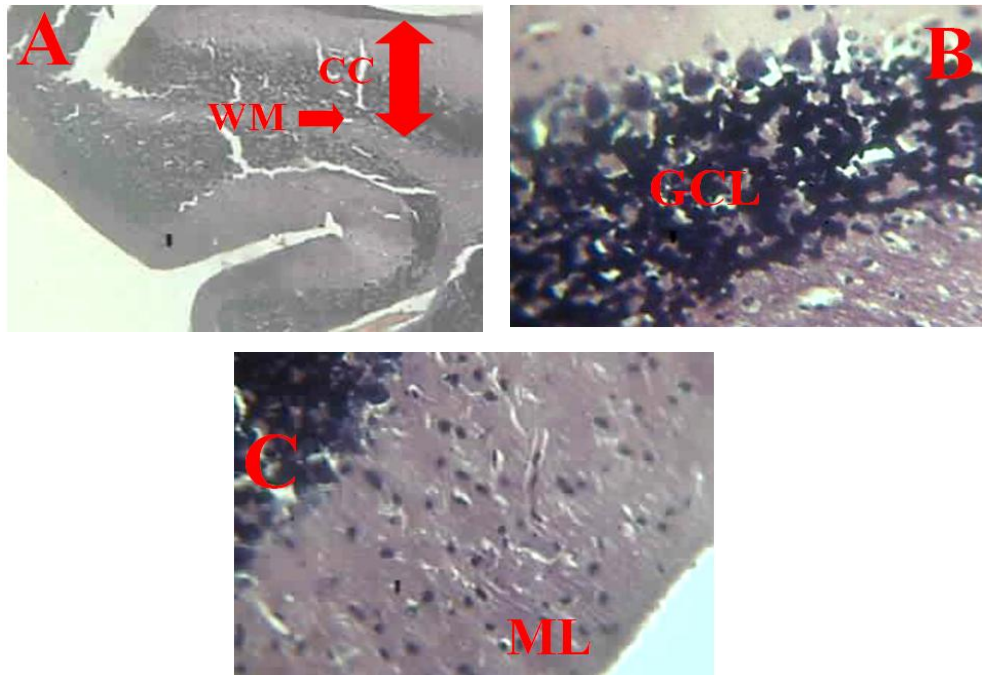


Fig. 6. Photomicrograph of the cerebellum of the Group F animals that were administered caffeine and cannabis using haematoxylin and eosin staining technique. Cortical histo- and cytoarchitecture is largely preserved
CC= Cerebellar Cortex; WM= White Matter; ML= Molecular Layer; PC= Purkinje Cell; GCL= Granular Cell Layer

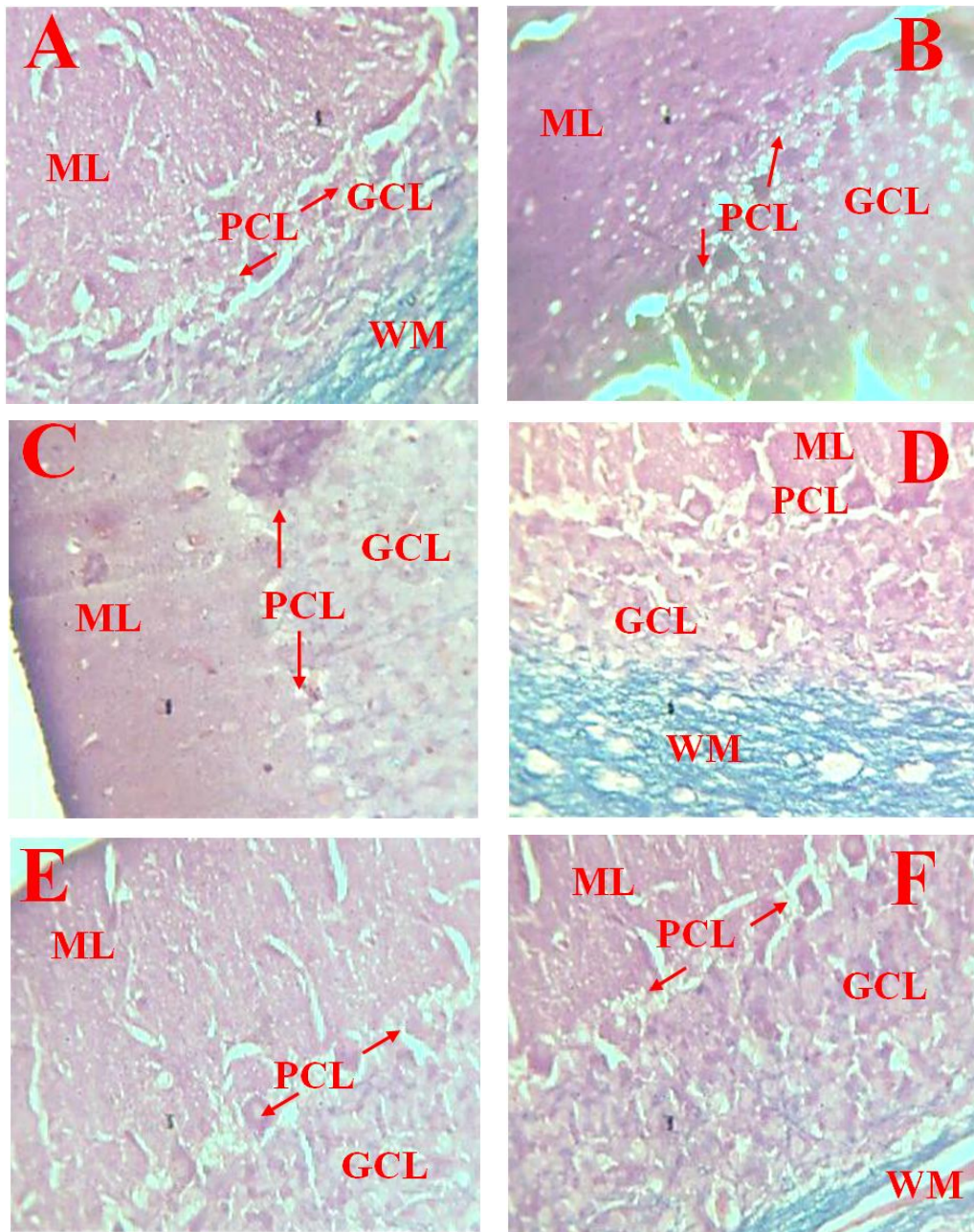


Fig. 7. Photomicrographs of Groups A – F experimental animals showing the myelin integrity of the cerebellar cortex; using the Luxol Fast Blue staining technique and there no signs of extensive disruptions to it due to the treatments given the experimental animals

Low dose of cannabis was administered to the Group D animals; though the cerebellum especially in terms of its architecture are still largely preserved the Purkinje cells are sparsely distributed; morphologically, they are mildly distorted and relatively smaller. These observations suggest that cannabis produce this

effect on this cells this effect might also affect the pattern of communication of these cells possibly by compromising their elaboration of their fibre. This effect appears aggravated as observed when high dose cannabis was administered to the animals in Group E. This observation suggests that cannabis produced deleterious

effects on the cerebellum and the severity of the effects increased with dosage. Furthermore the Purkinje cells are the prominently affected cells. Cannabis therefore has the potential to affect cerebellar cortex cells; particularly the Purkinje cells and deform them morphologically. These effects might alter the primary neurological functions of these cells such as their involvements in motor activities. These Purkinje cells are central to cerebellar cortex circuit [21]. In human studies, cannabis has been reported to have potentials to alter patterns of neurological development in adolescents [22]. Variations in specific brain region volumes are also reported [23] and structural integrity are also reported [24]. A more specific report showed that cannabis use is associated with variations in cerebellar volumes [25]. Interestingly, cerebellar injuries are associated with impairment in learning tasks and complex nonmotor processing [26]. It is interesting to note that most reports on adolescent cannabis effects that emphasized functional observations basically without paying much attention to possible underlying structural factors. Some of the effects of cannabis on adolescent animal models' brains include poor cognitive performance [27]; relatively poor social and grooming behaviours [28]; deficits in objects recognition [29,30]

When caffeine and cannabis were combined and administered to the animals in the Group F; the cerebellar cortex was largely preserved in this group. It is important to note that this particularly group received the low dose of caffeine combined with the lower dose of cannabis. It has been shown that caffeine did not cause cellular morphological alterations. Cannabis on the other hand at the lower dose also only caused mild alterations. Hence the combination of these two psychoactive substances, especially at the doses employed might not produce extensive damage to the cerebellum.

3.2 Myelination and Fiber Demonstration

Neurons communicate by virtue of their axons and dendrites. Axons are largely myelinated; the myelin or myelination pattern integrity in a brain tissue could therefore provide insight into the communication integrity of the constituent neurons. The Luxol Fast Blue staining technique demonstrated the cerebellar cortical myelination integrity as well as the integrity of the central white matter. Observations show that there is no extensive disruption to the myelin material across the cerebellar cortices of the animals across the

various groups. This again supports the fact that the administered substances are not basically toxic especially at the doses being employed; though they have been established to be psychoactive agents capable of influencing the functions and attributes of the various parts of the brain. A close look at the groups that were administered cannabis (Groups D and E) however shows less intact myelin relative to the other groups (Fig. 7D and E). This observation suggests that cannabis could alter neurocortical myelination integrity. Cannabis had been previously reported to influence myelination; however, it was said to have no established pathological implications [31,32]. This again, is in line with the morphological observation that the Purkinje cells were morphological affected and deformed. It is important to note that the molecular layer of the cerebellum is a field of projection for a number of Purkinje cells processes or dendrites and as such, the myelin, in addition to the morphology of these cells, might have been negatively affected by cannabis. Damage to cerebral cortical fibres may be associated with motor activities since damage to cerebellar tissue would ordinarily negatively affect fine movement, equilibrium, posture, and motor learning [3].

4. CONCLUSION AND RECOMMENDATION

Caffeine did not cause any observable morphological damage to the cerebellar cortex relative to this overall histoarchitecture and cytological elements. Cannabis caused distortions to the Purkinje cells and myelin integrity and the effects increased with dosage. It is therefore recommended that attention should be paid to high cannabis use because it could prove harmful to the cerebellar functions which could affect the quality of life.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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