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Cannabis Effects on the Visual Cortex Hisotarchitecture of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JOO and FOA designed the study. Authors ABOD and ATO supervised the research. All authors contributed to writing the protocol and the first draft of the manuscript. Authors SYO and AJO managed the analyses of the study. Authors JOO and FOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Cannabis is the most widely abused illegal substance in many countries of the world presently. It acts on the higher nerve centers and produces a feeling of intoxication with hallucination. Its main component, delta-tetrahydrocannabinol, produces the 'high' feeling that most users crave. Adolescence is the age of continued neuromaturation and most users experiment at the adolescent age. The aim of this research is to evaluate the histological effects of *Cannabis sativa* on the visual pathway of adolescent Wistar rats. A total of twenty-four (24) adolescent Wistar rats were recruited primarily for this study. They were randomly divided into four groups of six rats each labelled A, B, C, D which include the Control, Low Dose Group, Medium Dose Group and High Dose Group respectively. Administration lasted for 21 days with the Control group being administered pelletized rat chow and clean water *ad libitum*, the Low Dose group being administered 150 mg/kg body weight of the rat, the Medium Dose group being administered 250 mg/kg body weight of the rats and the High Dose group being administered 500 mg/kg body weight of the rats. At the end of the

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21-day administration, the animals were sacrificed and the brain tissue specimens were excised and processed. The general histological demonstration of the superior colliculus, lateral geniculate body and the visual cortex was done using the H & E and Luxol Fast Blue Staining Techniques. There were observable effects of Cannabis on the body weight after administration. There were no serious morphologic changes in the organ weight in Groups B and C, but there were indications of such in Group D. The observable histologic effects of cannabis on the visual cortex are specific on neuronal morphology, spatial distribution of neurons and glia and neurophil integrity and this is dose-dependent.

Keywords: Brain; visual pathway; cannabis; adolescence; effects.

1. INTRODUCTION

In the human, the primary visual cortex is situated along the superior and inferior banks of the calcarine fissure, corresponding to area 17 of Brodmann. This area often is called the striate cortex because of a prominent white band of myelinated fibers known as the stria of Gennari, which forms horizontal connections within the cortex. In humans, the striate cortex envelops the posterior pole of the hemisphere, extending laterally about 1.5 cm. rostrally and medially, V1 extends anteriorly beyond the juncture of the parieto-occipital and calcarine fissures, especially ventrally [1,2]. The optic radiations from the dLGN provide the main afferent connections to area V1. The three main outflow pathways from the retina—parvocellular, magnocellular, and konio- cellular remain distinct at the dLGN, but there is some convergence of these outflow in area V1 [3,4]. There are also inputs to area V1 from the pulvinar and other cortical regions.

Visual cortical neurons are divided into two main morphologies (pyramidal and non-pyramidal), which forms the basis for the histologic segregation of the visual cortex into six laminae [5]. Layer 1, the most superficial layer, contains few neurons and is composed mostly of fibrillary astrocytes with some microglia and oligodendrocytes. Layer 2 contains small pyramidal cells, many with short axons or axons that ascend and split within layer 1 [6]. Layer 3 is traditionally defined as containing mostly medium-sized and small pyramidal cells, with granule cells more deeply. A more recent interpretation further divides layer 3 into four subregions: 3A, 3Bα, 3Bβ and 3C [7,8]. Large pyramidal cells of 3A project to V2 in the monkey[6]. Cells in 3Bβ project to area V2, while those in 3C project both to V2 and MT. In the macaque, layer 3 receives input from the intercalated layers of the dLGN [9].

Layer 4 is a relatively large lamina that is the primary recipient area of the geniculate calcarine projection, which mostly synapses on stellate cells that have a uniform, radial topography. A recent revision of visual cortical architecture divides layer 4 into three zones: 4α, 4ctr and 4β [7,8]. The traditionally described layer 4A, which contains large stellate cells with axons that descend to deeper laminae or enter the subcortical white matter, expresses in humans monoclonal staining patterns not found in nonhuman primates, which suggests evolution of an enhanced interneuronal population [10]. The traditional layer 4B, recently considered to be 3C, contains mostly granule cells and has the stria of Gennari. The neurons of layer 4B, which predominantly receive input from the ‘‘M’’ pathway via layer (new classification), are mostly tuned for orientation, although a smaller population of cells are direction-selective [11-19]. These cells project mostly to area MT. Pyramidal cells in layer 3C receive M- and P-cell input and project mainly to area V2 [20-22].

Layer 5 contains pyramidal cells of various sizes, including the giant pyramidal cells of Meynert. Layer 5 (in the monkey) projects to the Superior Colliculus and pulvinar nucleus [20]. .Layer 6 contains medium-sized neurons [23], most of which project as a feedback pathway to the dLGN [20,24]. Layer 6 of macaque consists of three sub layers, only two of which project to the dLGN. The upper tier projects exclusively to parvocellular layers, while the lower tier projects to parvocellular and magnocellular layers of the dLGN [25]. The majority of striate cortical cells receive their (supra- threshold) excitatory input from either the ‘‘P’’ or ‘‘M’’ pathway via the dLGN. This description of parallel inputs, however, too greatly simplifies the reality. In macaquemon-keys, a substantial proportion (at least 25%) of area V1 neurons receive convergent input from two dLGN outflow pathways, usually the ‘‘M’’ and ‘‘P’’ pathways, and possibly from the koniocellular projections as well [21].

Cannabis is the most widely used illicit drug in the world [26]. Cannabis use was suggested to cause less efficient visual-motor function or visuospatial skills [27-30] and decreased visual processing speed [31]. These evidences points to the fact that cannabis use has effects on brain functions and performances, especially vision related activities. Also, the brain pattern of development may be altered by prolonged cannabis use [32]. A number of reports have stated cannabis effects to include reduction and increase in grey and white matter respectively in heavy cannabis users [33]; Others reported reduced whole brain volumes in individuals who started cannabis use in early teenage relative to those who started cannabis use above age 17 [34]. Interestingly other reports countered these claims and reported no significant variations in cerebral [35,36] and hippocampus volumes [36]. What is noteworthy here is that these results were imaging based with efforts concentrated on brain volume and atrophic changes rather than specific cellular morphological changes. The fact that human subjects were used also limited the extents of morphological analyses outside the bodily milieu. In few existing instances, cannabis extracts was reportedly toxic to cortical tissues at relatively high dose [37,38]. The current investigation however employed the use of histomorphological techniques to analyse observable changes in the visual cortex histoarchitecture and individual cells morphologies after exposure to various does of cannabis over a period of 21 days.

2. MATERIALS AND METHODS

The animals were kept in cages with constant supply of standard rat chow and water *ad libitum*. They were maintained at constant room temperature and 12 hours light/dark cycle. The animals were first allowed to acclimatize in a well aerated room with temperatures in the range of 20-25℃ and humidity of 40-45% for 7 days prior to the commencement of the experiment. A total of 24 adolescent wistar albino rats of both sexes were used for the experiment. They were purchased from the laboratory animal house of Babcock University, Nigeria. Average weight of rats at purchase is 86.27 g. Group A (n=6) Animals were given standard rat chow and clean water; Group B (n=6) Animals were given 150 mg/kg body weight of cannabis sativa.; Group C (n=6) Animals were given 250 mg/kg body weight of *Cannabis sativa* Group D (n=6) Animals were given 500 mg/kg body weight of *Cannabis sativa*. Animals were housed, handled and treated with adherehce to ethical and intitutional standatrd practices [39]; Ethical approval for research was granted by the Departmental Research and Ethical Comitteee [ANABU16/AD005].

Aqueous extract of *Cannabis sativa* leaves was prepared [40] and admisntred top the animal groups based on the designed regimen, daily though the orgastric route. Treatment asted 21 days and the animsl were sacrified by cervcial dislocation 24 hours after the last adminstration of the extract. Each rat was dissected and the brain tissue was surgically excised. Tissue preparation and analysises procedures included fixation, dehydration; with alcohol; Clearing; with xylene; embedding; with wax; blocking; sectioning; with a microtome; mounting; on the slide; dewaxing; with xylene; rehydration; with alcohol; staining; with haematoxylin and eosin [41] or and the Luxol Fast Blue staining technique [42]; cover slipping; photomicrography and photo micrographic analysis [43]. Represnetative photomicrographs of the visual cortex tissues were presented in the results sections as Figures.

3. RESULTS

Results of the experiment demonstrated using the H & E staining technique were obtained using the photomicrographic set. Representative photomicrographs were obtained to present and demonstrate the observable effects.Cannabis administration did not cause observable extensive cortical tissue distruption. Morphological abberations were however observed among the cortical neurons when animals were administered the highest dose of caffeine. Such effects could be attributed to the effects of cannabis on the cortical tissue. To this end, the observable effects on the cortex due to cannabis was neuoral morphological heterogeneity at high dose.

4. DISCUSSION

4.1 Histoarchitecture and Cellular Integrity

The photomicrographs in Figs. 1- 4 present the histological demonstration of the visual cortex of the experimental animals in groups A-D respectively (H & E). In Fig. 1 the general histoarhitecture of the visual cortex is normally demonstrated in the cross-section and the cortical layer is defined. At the higher

magnification, neurons and glia are normally demonstrated. The superficial layers (Fig. 1B) present few neurons in molecular layer and the granular cells with normal morphologies and spatial distribution, this is also true of the cells in the deeper cortical layers (Fig. 1C). These observations show that the Visual Cortex in these group of animals is normal and is suitable for a standard reference. Low dose of cannabis was administered to the animals in Group B; the visual cortex in this group is normally demonstrated in its cross-section (Fig. 2A). Superficial cells especially the granular neurons are prominently demonstrated and normal in morphology. The deeper cortical neurons especially the pyramidal cells (Figs. 2B and C) are also normally demonstrated. Generally, the pattern of spatial distribution of the cells as well as the glia is relatively normal in this group. The neuropil is also relatively intact. These observations altogether suggest that the administration of low dose of cannabis did not produce any observable deleterious effect either on general histoarchitecture, cell spatial distribution or morphologies in this group. There are therefore no deleterious effects. Thus, low dose cannabis use [≈150 mg/kg body weight] might not cause extensive structural damage or alteration to the visual cortex.

Relative to the Control Group A, the cross-section of the visual cortex in Group C is largely preserved in terms of the characteristic histoarchitecture (Fig. 3A). At the higher magnification, the cells of the superficial cortical layers are also largely unaffected in terms of their morphologies and spatial distribution (Fig. 3B).

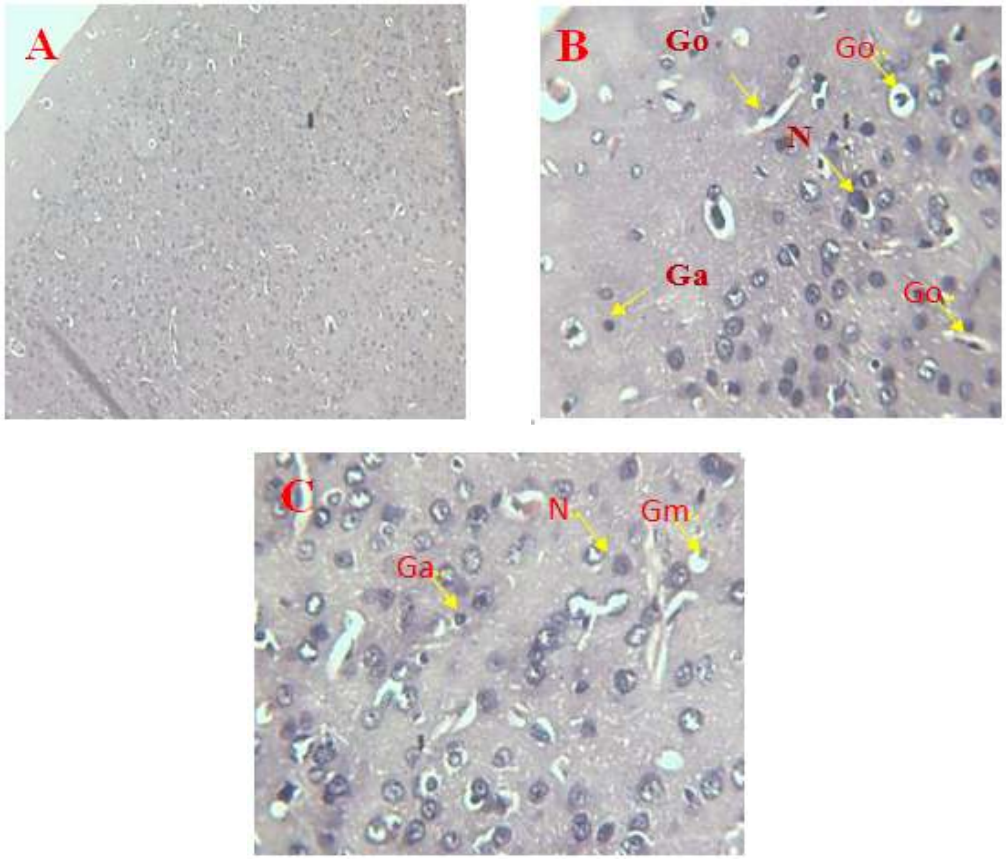

Fig. 1. Photomicrographs of the visual cortex of the control group demonstrating the cortical cross-section [A] and the superficial and deeper cortical cells respectively [B and C] [H&E]. Cortical histoarchitecture appears normal and suitable to serve as standard reference for the treated groups of animals

[N= Neuron; G= Glia- astrocyte; Go= Glia- oligodendrocyte; Gm= Glia- microglia]

However, certain neurons in the deeper cortical layers stain with varying intensity and some appear relatively heterogeneous Some other cells in this group also stain as dark or pyknotic neurons. This is characteristic of neurons that have been chemically assaulted or by other forms of trauma and it typically marks the beginning of the cascade of pathological response to assault by neurons [43]. Consequently, the administration of medium dose of cannabis to the animals in this group produce observably mild negative effects in these few neurons and this might compromise their neurologic integrity. Realtively higher caffeine dose would therefore cause structural changes to the visual cortex; and similar effects have been reprted [44-46].

The high dose of cannabis administered to the Group D animals also produced observable effects on the visual cortex in this group, though, the histoarchitecture is still largely preserved in its cross-section (Fig. 4A). Howver, the superficial granular cells are poorly demonstrated (Fig. 4B). In the deeper cortical layers, cells appear relatively heterogenous with a few having large peri-cellular spaces. These observations collectively suggest that the administered substance caused distortions of cell morphology and the surrounding neurophil in this group. Such effects are relatively more severe than those observed in the preceding Group C and this shows that the effects of the administered substance is dose-dependent. The inference from this obervation is that cannabis ingestion at

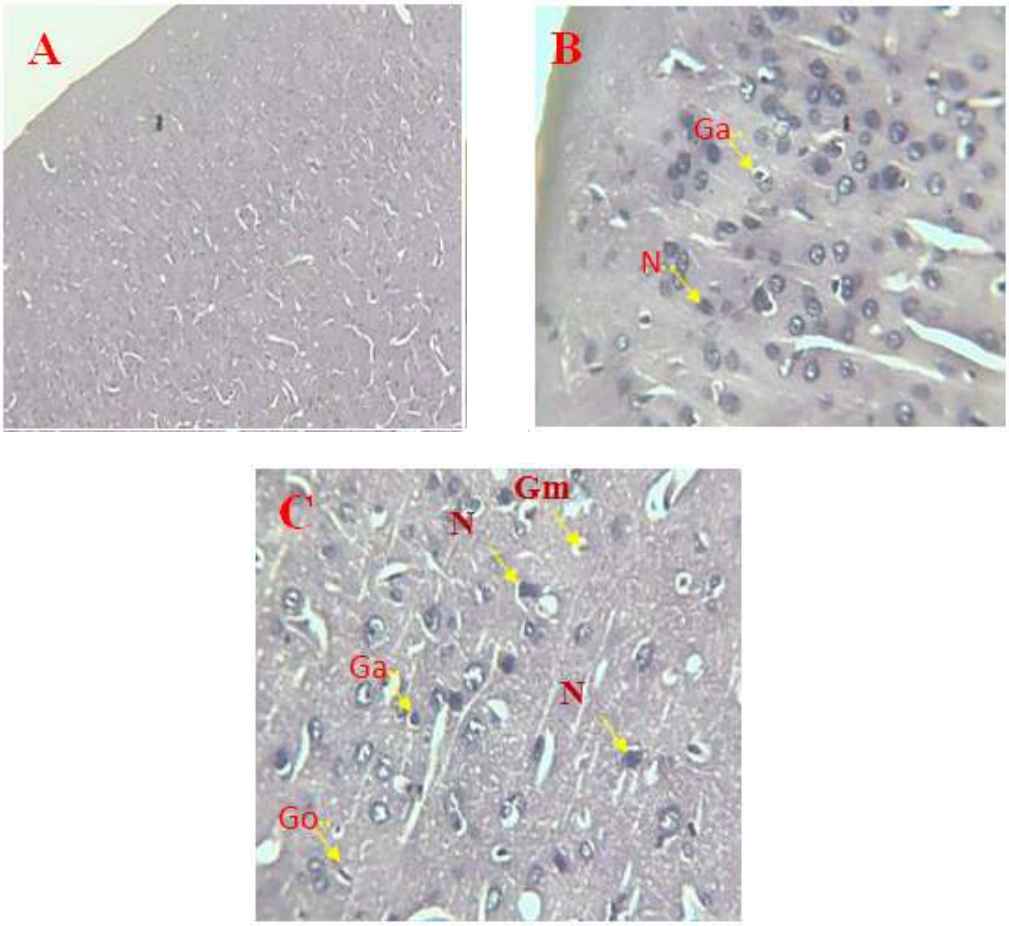

Fig. 2. Photomicrographs of the visual cortex of the low dose group demonstrating the cortical cross-section [A] and the cortical cells [B and C] [H & E]. Cortical histoarchitecture appears relatively normal and devoid of extensive disruptions or individual morphological aberrations

N= Neuron; G= Glia- astrocyte; Go= Glia- oligodendrocyte; Gm= Glia- microglia]

relativly hight dosage would cause detelrtous changes to the cerenbral cortex nurons, changing their mophologies grossly. This agrees with a number of reports on the potential toxic effects of cannabis to cotical tissue [47,48]; however, at relativly high doses. It also shows that cannabis effects on the brain cortex is dose depenedent and severity may incraese with dosage.Though many reports and review exist on the functional consequences of effects of cannabis on the brain [49-53]; Reports on brain and cortical structural changes are relatively inadequate presently and this reaffirms the relvenace of this article.

4.2 Neurophil and Myelination Integrity

The Luxol Fast Blue Staining Technique was used to demonstrate the myelination and neurophil integrity across the animal groups. The neurophil and pattern of myelination appear normal in the Control Group (Fig. 5A). The observation in Group B (Fig. 5B) is also similar to that of the control, showing that the neuropil is still largely preserved when the medium dose of cannabis was administered. Myelin was demonstrated in the Group C cortex, though there are still signs of mild localized disruptions (Fig. 5C). This might be due to the pyknotic condition of certain cells previously observed. High Dose of cannabis was administered to the Group D animals and the Luxol Fast Blue staining technique shows that relative to the neurophil, cells are less prominently demonstrated in the group. This, again, is attributable to the disrupted cortical tissue previously reported. Again this obervations support the accompanying cortical fibre poor myelination changes that reporteldy accompany cannabis use, especially during adolescence [48].

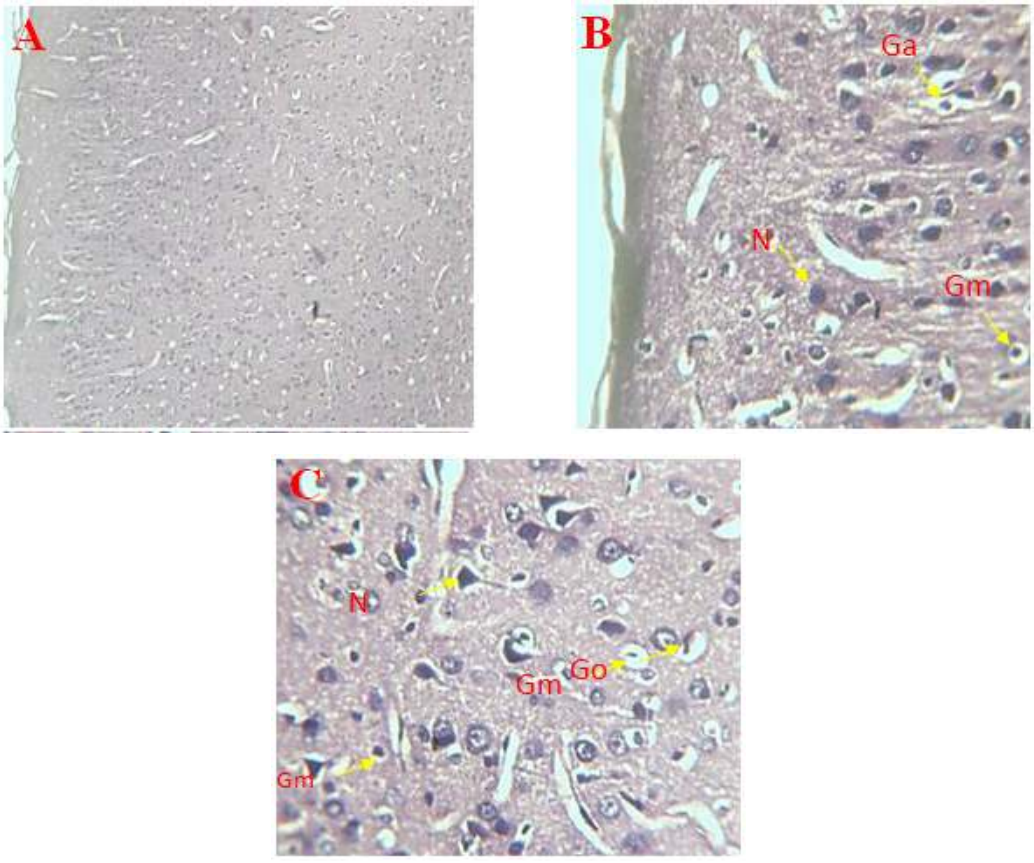

Fig. 3. Photomicrographs of the visual cortex of the medium dose group demonstrating the cortical cross-section [A] and the superficial and deeper cortical cells respectively [B and C] [H&E]. Cortical histoarchitecture appears normal. Neurons stain with varying intensity and some appear relatively heterogeneous

[N= Neuron; G= Glia- astrocyte; Go= Glia- oligodendrocytes; Gm= Glia- microglia]

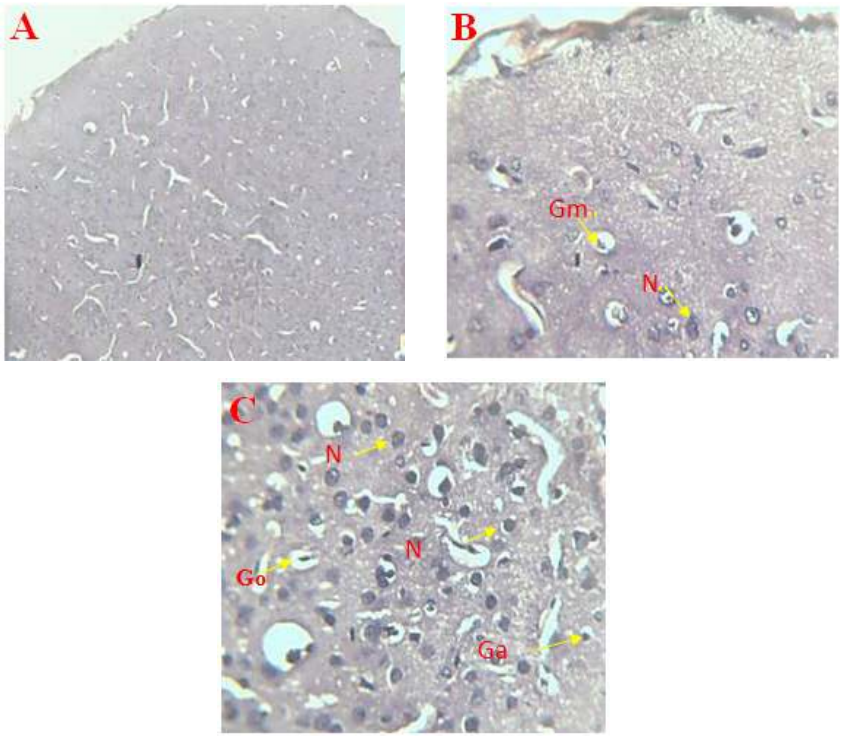

Fig. 4. Photomicrographs of the visual cortex of the high dose group demonstrating the cortical cross-section [A] and the superficial and deeper cortical cells respectively [B and C] [H & E]. Cortical histoarchitecture appears relatively normal; but neurons are heterogeneous in morphologies relative to one another

[N= Neuron; G= Glia- astrocyte; Go= Glia- oligodendrocyte; Gm= Glia- microglia]

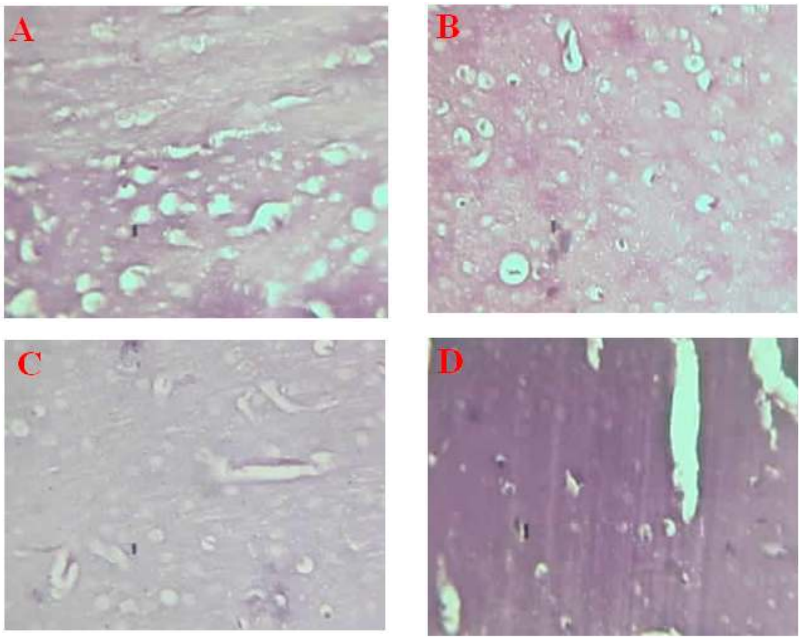

Fig. 5. Photomicrographs of the visual cortex of the control group [Luxol Fast Blue]

There are no substantial signs of extensive disruption to the visual cortex myelin integrity

5. CONCLUSION

Results from the histological osbervations of the visual cortex of the experimental animals showed that cannabis extract ingestion had mild effects on the ccortex. These effects were observed at the reltively high dose of cannabis; and these effects specifcally included morphological heterogeneity of certain neurons.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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