

Histomorphologic effects of *Cannabis sativa* on the brains of adult Wistar rats

ABSTRACT

Introduction: *Cannabis sativa* is one of the species of cannabis plant. The objective of this study was to determine the short- and long-term effects of *Cannabis sativa* on the brains of adult Wistar rats. **Materials and Methods:** Thirty (30) healthy adult Wistar rats of both sexes were used as experimental animals. The *Cannabis sativa* leaves obtained were extracted at the Laboratory Facility of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University, Abraka, Nigeria. The animals were grouped as follows: Positive control (one: Group I), negative control (one: Group II), and experimental groups (three: Groups III-V); different doses of the drug in volume were calculated (0.10 mL for acute study, 0.23 mL for subacute study, and 0.50 mL for subchronic study) and administered orally with the use of an improvised orogastric cannula. The animals were sacrificed and the organs harvested for manual tissue processing and hematoxylin-eosin (H&E) staining. The stained slides were interpreted with the aid of a light microscope and analyzed for histomorphological changes across the groups. **Results:** The results showed that the histomorphologic changes induced by *Cannabis sativa* in short- and long-term studies caused extensive cerebral gliosis in the brains of adult Wistar rats. **Conclusion:** It could, therefore, be deduced that there were both dose- and time-dependent toxic effects of *Cannabis sativa* on the experimental animals. *Cannabis sativa* was shown to cause marked neuronal changes in the brains of adult Wistar rats.

Key words: *Cannabis sativa*, cerebrum, gliosis, histology

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INTRODUCTION

Cannabis could be described as a dry, shredded mixture of the flowers, stems, leaves, and seeds of the cannabis plant. The plant has three different species including: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*. *Cannabis sativa* leaf is typically green, but brown if dried. All forms of cannabis contain delta-9- tetrahydrocannabinol (Δ^9 -THC) as the main psychoactive ingredient.^[1]

Previous research has shown that the brain is one of the sites of action of *Cannabis sativa*.^[2] Cannabinoid type 1 (CB1) receptors are distributed widely throughout the central, peripheral, and enteric nervous systems.^[3] Cannabinoid

type 2 (CB2) receptors are closely linked within the immune tissue, predominantly the spleen and macrophages.^[4]

In another report,^[5] it was shown that long-term, heavy *C. sativa* use was associated with gross anatomical abnormalities in two cannabinoid receptor-rich regions (the hippocampus and the amygdala) of the brain. Similar studies have shown that heavy, daily *C. sativa* use across protracted periods exerted harmful effects on brain tissue and mental health.^[6-9]

The report of another study showed that an acute dose of oral THC was associated with a decrease in threat-related amygdala reactivity, which suggests that cannabinoids have an inhibitory effect on threat-related amygdala reactivity.^[10]

The literatures suggest in general that long-term *C. sativa* use is associated with a wide range of adverse health consequences, especially on the brain.

This study was therefore aimed at determining the acute and chronic effects of *C. sativa* on the brains of adult Wistar rats.

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MATERIALS AND METHODS

Experimental design

Thirty (30) healthy, adult Wistar rats of both sexes were used as experimental animals.^[11]

Ethical considerations

Approval was obtained from the Research and Bioethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria, and the study was carried out in strict accordance with the guidelines for the care and use of animals for research.^[12]

Procurement of plant material and authentication

Permission was sought from the National Drug Law Enforcement Agency (NDLEA) at their head office at Ogwashi-Uku, Delta State. The leaves were also obtained from the NDLEA office and were authenticated at the Department of Botany, Delta State University, Abraka.

Method of *C. sativa* extraction

The agent *C. sativa* was extracted using standard methods^[13] at the Laboratory Facility of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University, Abraka.

Animal grouping and administration of plant extract

On transfer to the work area, the animals were allowed 14 days for acclimatization. The Wistar rats weighed 150-195 g and were divided at random into five groups of 6 animals each of both sexes, as follows: Group I (positive control), Group II (negative control), Group III (experimental group 1), Group IV (experimental group 2), and Group V (experimental group 3). The drug was administered to the groups via oral route using improvised orogastric cannula as follows: Rats in group I received normal saline for 42 days; group II 0.10 mL (acute), 0.23 mL (subacute), and

0.50 mL (subchronic) 5% ethanol for 7 days, 21 days, and 42 days respectively; group III 0.10 mL/day of 5% ethanol extract of *C. sativa* for 7 days (63.5 mg/kg/day) for acute study; group IV 0.23 mL/day (127 mg/kg/day) of 5% ethanol extract of *C. sativa* for 21 days for subacute study; and group V 0.50 mL/day (254 mg/kg/day) of 5% ethanol extract of *C. sativa* for 42 days for subchronic study. The animals were housed within the facility and maintained on standard rodent pellets and water *ad libitum*.

Animal sacrifice and harvest of selected organs

After the experimental periods, rats in each of the groups were weighed and then sacrificed by cervical dislocation. Harvest of the organs was made on the same day; the harvested organs were placed in universal bottles that were labeled accordingly and preserved with 10% formal saline for 48 h. The tissues were then grossed and placed into tissue cassettes that had been labeled, and thereafter the tissues were processed manually as prescribed previously.^[14]

The stained tissue images were captured using the digital camera and microscope ScopeTek DCM500 connected to the universal serial bus (USB) port of a computer. The stained slides were interpreted with the aid of a light microscope and analyzed for histomorphological changes across the groups.

Inclusion/exclusion criteria

The experimental animals were randomly chosen based on the following criteria during and after the period of acclimatization: Rats that weighed 150-195 g and had normal feeding patterns were included, while rats that had physical sign of sickness or weakness were excluded.

RESULTS

In Plates 1 and 2, sections through the cerebrum for the positive and negative control groups are shown.

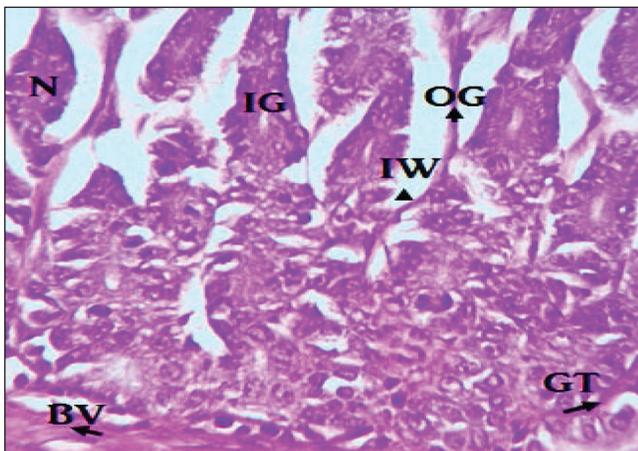


Plate 1: Rat cerebrum — Positive control (Group I) (H&E ×100) (N = Neuron; OG = Outer grey matter; IW = Inner white matter; IG = Innermost grey matter; GT = Glia tissue; BV= Blood vessels)

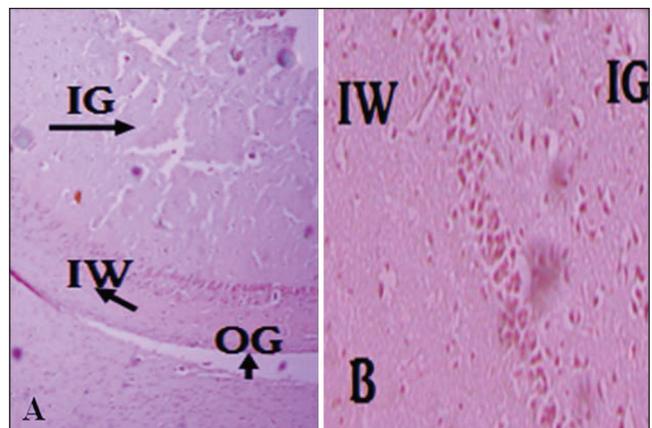


Plate 2: Rat cerebrum — Negative control (Group II) (A) H&E ×40 (B) H&E ×100 (OG = Outer grey matter; IW = Inner white matter; IG = Innermost grey matter)

Extensive gliosis is seen in all the experimental groups [Plates 3-5].

DISCUSSION

The results of the study as seen in the experimental groups were both dose- and time-dependent. Factoring in normal

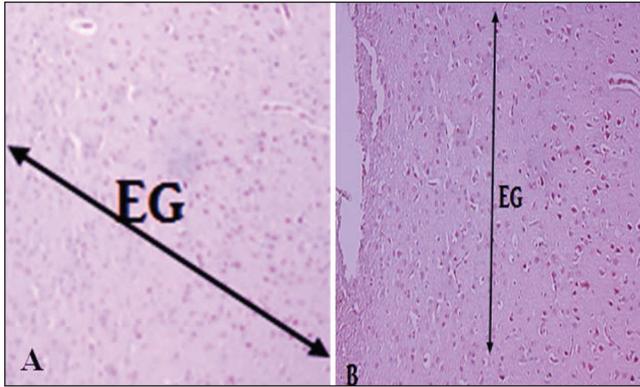


Plate 3: Rat cerebrum — Group III (A) H&E ×40 (B) H&E ×100 (EG = Extensive gliosis)

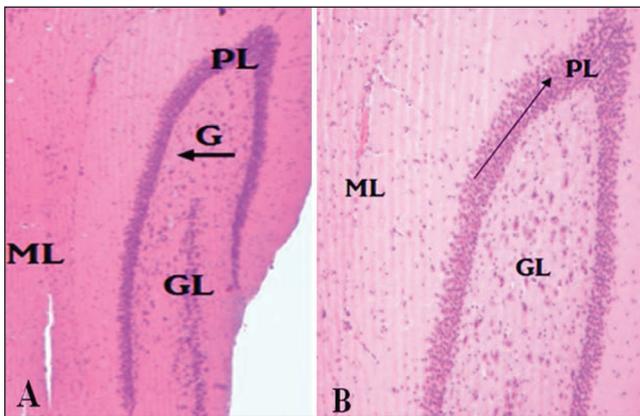


Plate 4: Rat Hippocampus - Group IV (A) H&E ×40 (B) H&E ×100 (G = Gliosis; PL = Plexiform layer; GL = Granular layer; ML = Molecular layer)

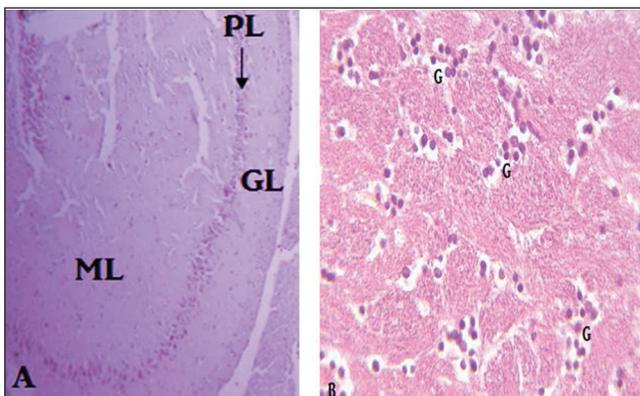


Plate 5: Rat hippocampus — Group V (A) H&E ×100 (B) H&E ×400 (PL = Plexiform layer; GL= Granular layer; ML = Molecular layer; G = Gliosis)

body adaptations, it was imperative to increase the dosage with time. An oligodendrocyte can be described as a type of glial cell that generates and maintains the formation of myelin around the axons of large neurons in the central nervous system (CNS), allowing for rapid transmission of neural signals.^[15] The presence of oligodendroglia indicated a proliferation of oligodendrocytes, which suggested damage of or injury to the CNS; this was in agreement with previous findings.^[5] It is noteworthy that several different patterns of oligodendrocyte injury/reaction may occur depending on the mechanism of insult, though some oligodendrocytes could be lost through necrosis or apoptosis, while others may survive and form part of the glial scar along with myelin debris.^[16] Oligodendrocyte precursor cells could also be affected by CNS insult and therefore be recruited to demyelinated areas within 1 week following traumatic injury. This may further suggest that the degeneration of axons due to trauma (such as the effect of *C. sativa* on the CNS) could result in the degeneration of the myelin sheath.

Moreover, cannabinoids have been known to act on specific neurotransmitters in certain memory regions of the brain, thereby causing memory loss.^[17,18] Glutamate, dopamine, and acetylcholine are three neurotransmitter systems that are thought to play a role in meditating the memory effects of cannabis. Consequently, *C. sativa* acts by reducing the glutamate levels by possibly using presynaptic mechanisms, activating dopamine receptor (D1) in the prefrontal cortex, and selectively modulating the activation of the cholinergic neurons in the septal-hippocampal pathway.^[19] *C. sativa* resulted in severe brain injury in the index study, especially in the cerebral region, resulting in gliosis, which is known to involve alterations in cellular activity, potentially creating widespread effects on neurons as well as other nonneural cells and resulting in impairment of several functions.^[20,21]

CONCLUSION

This study has clearly demonstrated that acute and chronic exposure of experimental rats to orally administered *Cannabis sativa* may result in marked neuronal damage.

Recommendation

More studies should be conducted to identify the molecular target of gliosis, knowing which could improve healing in all types of injury.

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