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HISTOARCHITECTURAL ORGANIZATION OF THE VISUAL SYSTEM OF MALE RATS FOLLOWING ORAL ADMINISTRATION OF CRUDE AQUEOUS LEAF EXTRACT OF CANNABIS SATIVA

Tijani AA[1], Adekomi AD[2, 3], Oyesomi TO[2], Fawole OB[4]

1. Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, Osogbo, Osun State, Nigeria
2. Department of Anatomy, College of Medicine, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria
3. Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
4. Department of ENT Surgery, LAUTECH Teaching Hospital, Ogbomoso Oyo state, Nigeria

Corresponding Author: TIJANI AA

Email: ahmad.tijani@uniosun.edu.ng

ABSTRACT

Aim: This study was to elucidate some of the effects of oral administration of *Cannabis sativa* on the visual system of male Wistar rats.

Methods: 12 adult male Wistar rats were used for this study. The rats were distributed into two groups (A and B). The rats in group A (treatment group) were administered with 300 mg/kg body weight of *Cannabis sativa* while the rats in group B (control group) were administered with equal volume of phosphate buffered saline. The duration of administration was 21days. The rats were sacrificed using cervical dislocation 24 hours after the last administration. The brains were excised and fixed in 10% formol calcium. 72 hours after fixation, right occipital cortex, right lateral geniculate nucleus and right superior colliculus were excised respectively for histological processing and sections stained with H&E.

Results: Microscopic observations revealed alterations in the histoarchitecture of the organs of visual system of the rats in the treated group compared with the rats in the control group with preserved histological outline.

Conclusion: Oral administration of *Cannabis* on the visual system of male Wistar rats caused degeneration in the neurons of occipital cortex, right lateral geniculate nucleus and right superior colliculus of Wistar rats.

Key words: Drug abuse, Vision, Cannabis sativa, Brain

INTRODUCTION

Cannabis, indigenous to central South Asia (ElSohly, 2007), is found to have occurred as long ago as the third millennium B.C as indicated by Charred Cannabis seeds found in ritual brazier at an ancient burial site in present day Romania (Rudgley, 1998). It is a coarse rangy, annual plant that grows 1.8-3.7 m in height with palmate leaves divided into 3-7 harrows and about 5.2-7.6cm long. Its stems are rough with fibrous inner bark. It is normally a dioecious species, with male and female flowers on separate plants, but sometimes bisexual plants occur (Steve, 2005). It thrives on rich, fertile, neutral to slightly alkaline, well

drained silt or clay loams with moisture retentive sub soils and is reported to tolerate annual precipitation of 3 to 40 dm, annual temperature of 26^0C to 27^0C and PH of 4.5 to 8.2. (Duke,1978). Over 60 cannabinoids have been isolated in the plant, but delta-9-tetrahyrocannabinol (THC) appears to be the major psychoactive ingredient (Harvey, 1999). Some of the other cannabinoids are cannabinol (CBN) and tetrahyrocarnabivarin (THCV). Duke in 1985 reported that 100g of dry seed of cannabis sativa is composed of 487 calories, 0% water, 31.4g protein, 29.6g fat, 31.9g carbohydrate, 23.5g fibre and 7.1g Ash, 139mg calcium, 1123mg phosphorus, 13.9mg Iron, 518mg, vit A, 0.37mg Thiamine (B_1), 0.2mg riboflavin (B_2), 2.43MG Niacin. Cannabis sativa is commonly called Indian hemp or Marijuana and popularly known as "igbo" in Yoruba language. It acts almost entirely on the higher nerve centers (Cooper and Johnson, 1984) and can produce an exhilarating intoxication with hallucination (Margaret, 1995). It was reported to be one of the most commonly abused illicit drugs in the world with over 83 million individuals in the United States having used cannabis at least once in their life time (NHSDA, 2002). While abundant experimental studies have illustrated the deleterious effects of cannabis use on cognitive functions such as memory and attention (Kanayama et al., 2004; Kempel et al., 2003; Skosnik et al., 2001; Solowij et al., 1995), very few laboratory studies have examined the effect of cannabis use on the visual system. It was reported to have produced toxic effects on the neurons of the visual cortex in rats (Tijani and Adekomi, 2011) and modulated sensory/perceptual function in the visual system (Patrick et al, 2006). Its psychoactive property made it a widely used street drug in Nigeria despite the legal implication of its possession and use. The visual cortex (VC), lateral geniculate body (LGB) and superior colliculus (SC) constitute the intracranial visual relay centers. In mammals, the two strongest pathways linking the eye to the brain are those projecting to the LGB, and to the SC (Goodale and Milner, 1992). The primary visual cortex surrounds the calcarine fissure, a horizontal fissure in the medial and posterior occipital lobe (Carlson, 2007). Each primary visual cortex receives information directly from its ipsilateral lateral geniculate body and transmits information to two primary pathways called dorsal and ventral streams (John, 2006). The visual cortex detects the orientation of lines and borders (Inderbir, 2007). The LGB is the primary processing centre for visual information received from the retina of the eye. It is found inside the thalamus of the brain and receives information directly from the ascending retinal ganglion cells via the optic tract and from the reticular activating system. Neurons of the LGB send their axons through the optic radiation directly to the primary visual cortex. In addition, the LGB receives many strong feedback connections from the primary visual cortex (Huerta and Harting, 1984). The general function of the superior colliculus is to direct behavioral response towards specific point in egocentric space. In primates, the superior colliculus has been studied mainly with respect to its role in directing eye movements. Visual input from the retina or command input from the cerebral cortex, create an event of activity in the tectal map, which if strong enough induces a saccadic eye movement (Dean et al, 1989). Even in primates, however, it is also involved in generating spatially directed head turns, arm-reaching movements, and shift in attention that do not involve any overt movement (Wyllie, 1980). Much has been documented about the physiological effects of cannabis on the various parts of the brain (Yucel et al, 2008; Quickfall, 2006; Block et al, 2002; Bolla et al, 2002; Solowij et al, 2002; Pope et al, 2001; Block et al, 2000; Solowij, 1998). The dart of research reports of cannabis effects on the intracranial visual nuclei, despite its well documented effect as a substance of visual hallucination informed the conception of this study. This preliminary study aimed at studying the anatomical effects of medium dose orally administered aqueous leaf extract of *C. sativa* on the intracranial visual relay centers of male Wistar rats using basic histological technique.

MATERIALS AND METHOD

Plant Extract

Six hundred grams of dried leaves of *Cannabis sativa*, obtained from the Kwara State Command of Nigerian Drug Law Enforcement Agency (NDLEA), Ilorin, Nigeria was milled to obtain a fine powder. 100 g of the powder was dissolved in

1000 ml of distilled water for 72 hours and filtered after 72 hours with Whatman's No 1 filter paper to yield 800 ml of filtrate. The filtrate was oven-dried at a temperature of 60°C for 7days to obtain a deep brown paste of 10 g which was dissolved in 50 ml of phosphate buffered saline to make a 200 mg/ml aqueous solution of *C. sativa*.

Animal Care and Treatment

All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institute of Health (1985). Twelve adult male Wistar rats with average weight of 200 g were used in this study. They were reared in the animal holding of the College of Health Sciences of Osun State University, Osogbo, Nigeria. They were fed with standard rat diet purchased from Kilekun Animal Feed and Concentrate, Polytechnic Road, Ede and were given water *ad-libitum*. They were kept in standard laboratory metallic cages in groups of three, cared for under standard laboratory conditions of good lighting, moderate temperature and adequate ventilation and were weighed routinely. Rats in group A were treated with 300 mg/kg body weight (0.3 ml) of *C. sativa* (Tijani and Adekomi, 2011) for 21 days. Rats in group B (control group) received equal volume of phosphate buffered saline for the same number of days. Administration was done orally with the aid of orogastric tube at 07.00 hour each day. Rats were sacrificed by cervical dislocation 24 hours after the last administration while brain tissues were carefully removed from the skull and fixed in 10% formol calcium for 72 hours, after which the right occipital cortex, right lateral geniculate nucleus and right superior colliculus were excised separately for histological (H&E) processing. The sections of visual cortex, lateral geniculate body and superior colliculus were prepared and slides examined using the Olympus binocular light microscope (XSZ-107BN, No071771). The photomicrograph of each slide was taken with a Samsung Digital Camera (Digimax i6 PMP, Samsung #11 PMP).

RESULTS

Histological sections of the visual cortex of the rats in the treated group showed vacuolations of the neuron signifying disruption in the histoarchitecture of the visual cortex (Plate 1a) when compared with the histological section of the rats in group B which has no altered histological profile signifying a well preserved histological profile (Plate 1b). Sections of the LGB of rats in group A (Plate 2a) showed vacuolations in the stroma of the cells. This may confer adverse effects on the functional integrities of the neurons in the LGB of the rats in the treated group. However, when this was compared with the sections of the LGB in group B rats, it was observed that the LGB in group B has a well preserved histological outlines (Plate 2b). Furthermore, the sections of the superior colliculus of the rats in group A also showed vacuolations of the neurons (Plate 3a) while the section from rats in group B have intact neurons with well-preserved cytoarchitectural profile (Plate 3b).

DISCUSSION

Neuronal degeneration or cellular damage in neurons has been reported to result in cell death. Cell death could be apoptosis or necrosis, which differ morphologically and cytochemically (Farber et al, 1981). The severity of the insults is proportional to the rate of progression of neuronal injury. The principle holds true for toxicological insults to the brain and other organs (Martins et al, 1978). The prime candidates for inducing the massive cell destruction observed in neurodegeneration are neurotoxins (Waters, 1994). This study showed some disruptions in the organs of visual system of rats administered with 300 mg/kg body weight of *C. sativa* for 21days. The result confirmed previous studies indicating that *C. sativa* has a complex effect on the brain (Nava et al, 2000; Hayatghaibi and Karimi, 2007; Muktar and Elbagir, 2011; Tijani and Adekomi, 2011). According to our earlier study, 21day oral exposure of Wistar rats to cannabis resulted in various histopatological effects such as perinuclear spaces, vacuolations and widely affected Nissl substance on the visual cortex of Wistar rats (Tijani and Adekomi, 2011).

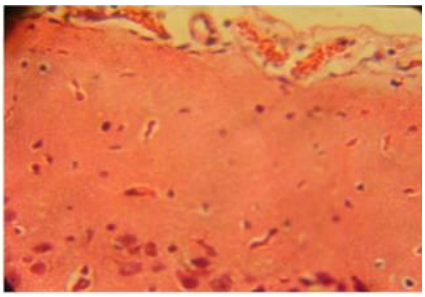

Plate 1a: Section of the visual cortex of the animals treated with *cannabis sativa* showing vacuolation of neurons, glial cells and pyramidal cells. The neurons appear sparsely stained (H&E x 520)

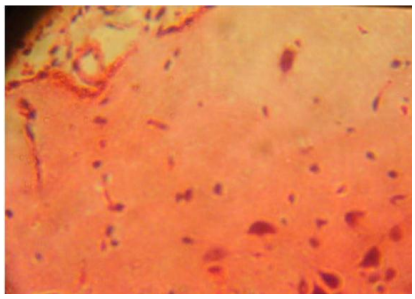

Plate 1b: Section of the visual cortex of the animals in the control group. There were no vacuolations in neurons, glial cells and pyramidal cells (H&E x 520

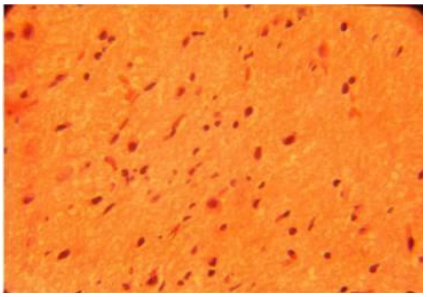

Plate 2a: Section of lateral geniculate body of the animals treated with Cannabis sativa showing vacuolations in the stroma (H&E X 520)

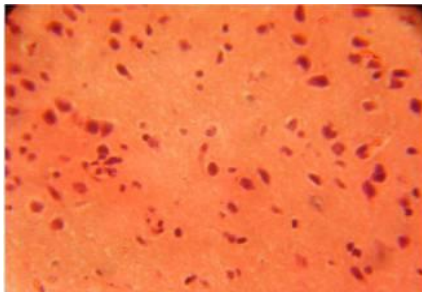

Plate 2b: Section of lateral geniculate body of the animals in the control group with well preserved histological outlines (H&E X 520)

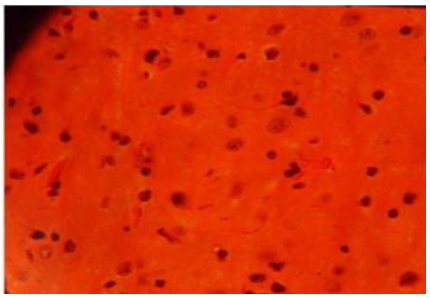

Plate 3a: Section of superior colliculus of a rat treated with Cannabis sativa showing vacuolations of neurons (H&E X520)

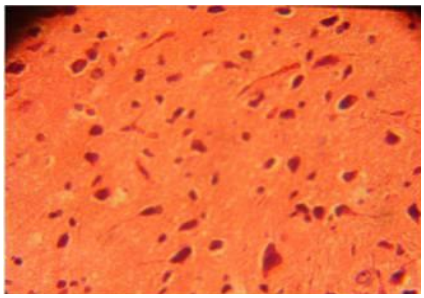

Plate 3b: Section of superior colliculus of a rat in the control group showing intact neurons with preserved cytoarchitectural profile (H&E X520)

Histological sections of the visual cortex of rats treated with cannabis in this current study showed similar histopathological profiles to our previous work (Tijani and Adekomi, 2011). The work of Sarne and Keren (2004) showed that chronic cannabinoids exposure induces long-lasting impairment of learning and memory, which was accompanied by morphological damage to the brain. It also showed that cannabinoids in high doses through excessive secretion of glutamate is neurotoxic. Allyn (2004) showed numerous CB1 receptor-mediated effects have been observed, ranging from modulation of nociception and glutamate transmission to inhibition of long-term

potentiation. However, reliable evidence about the validity of this claim is not available. According to Tehranipour and Ebrahimpour (2009), THC is capable of causing some alterations in hippocampus neuronal structure and processes. Vacuolations in the stroma shown in the H&E stained sections of the LGB from the rats in group A indicated neuronal degeneration and progressive cell death. The reduced cellular proportion in the group compared to group B rats is also in line with the deleterious effects of the plant extract on the LGB. In the SC, vacuolation of the neurons and stroma in sections from group A rats with reduced proportion of neurons in the group showed that cellular damage occurred in the superior colliculus of the rats compared with the well preserved histological outline of the SC in the group B rats. Slides from group A rats were sparsely stained, indicating that the cellular damage effect is pronounced in the rats. The outcome of this study could be attributed to heavy metals like cadmium, arsenic and lead which are present in *C. sativa* (Smith et al, 1997; Satarug et al, 2004). These heavy metals could replace the trace elements from antioxidant markers and may also deplete the enzyme activities in the brain (Sulochana et al, 1998), thereby resulting into a compromise in the morphological, histochemical and cytochemical characteristics of the visual system. If there is any alteration in the critical balance in the normal profile of the neurons in the visual system, an increase in the levels of reactive oxygen species and cellular damage may occur (Ramesh et al, 2007). The observations made in this study are in compliance with the study of Hall and Solowij (1998). Oral administration of aqueous leaf extract of *Cannabis sativa* to adult Wistar rats at a dose of 300 mg/kg body weight on a daily basis for 21days produced some histological changes in the visual system of the rats. These histological changes are all indicative of necrotic process in the tissues with the involvement of lysosomal destruction. *Cannabis sativa* is seen from the research work to be neurotoxic to the visual system at the dose administered.

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