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Original Research Article

Reversible Effect of Marijuana on the Rat Hippocampal Tissue

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ABSTRACT

Introduction: THC (Δ – 9 – tetrahydrocannabiol) is considered the active ingredient of Marijuana and it has a neurotoxic effect on one of the most sensitive brain regions, hippocampus.

Objective: The aim of the present work was to assess whether this neurotoxic effect is reversible or not.

Methods: This study was conducted on 60 albino rats randomly subdivided into Group I: Control group, Group II: received 10 mg/kg Δ – 9 – tetrahydrocannabiol for 5 days then sacrificed on 5th day, Group III: received 10 mg/kg Δ – 9 – tetrahydrocannabiol for 5 days then sacrificed 16 days after the last dose. Brain tissue was removed, hippocampus was dissected and examined by light microscope then total number of neurons was determined.

Results: The present study showed that repeated Δ THC doses resulted in decreases in neuron cells of rat hippocampus. While Group III, in which rat's hippocampus was studied 16 days after last dose of Δ THC, the average number of cells was statistically lower than the control group but higher than Group II.

Conclusion: Repeated doses of Δ THC was shown to cause decreases in rat hippocampus cells in the present study. In addition, it was also found that cell numbers increased after the Δ THC administration was stopped.

Keywords: Δ – 9 – Tetrahydrocannabiol, Marijuana, Neurotoxic, Hippocampus.

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INTRODUCTION

THC (Δ – 9 – tetrahydrocannabiol), is the active ingredient in Marijuana which is a commonly abused drug(Wiley et al., 2013). Cannabis sativa (Marijuana) usually is smoked or might be mixed in food or tea. Marijuana smoke has a pungent and distinctive, usually sweet-and-sour odor.[1]

The immediate effect of marijuana smoking continue up to one or three hours, but, If marijuana is consumed orally, there will be slower short-term effects, usually in one hour, and last for 4 hours.[2] Someone who smokes marijuana regularly may have many of the same respiratory problems that tobacco smokers do, such as daily cough, more frequent acute chest illnesses, a heightened risk of lung infections, and a greater tendency toward obstructed airways. Cancer of the respiratory tract and lungs may also be promoted by marijuana smoke.

Marijuana has the potential to promote cancer of the lungs and other parts of the respiratory tract because marijuana smoke contains 50 percent to 70 percent more carcinogenic hydrocarbons than does tobacco smoke.[3] Marijuana's damage to short-term memory seems to occur because THC alters the way in which information is processed by the hippocampus, a brain area responsible for memory formation.[4]

Hippocampus, considered to be the center of long-term memory in central nervous system, is a region of the brain sensitive to neurotoxic drugs.[5]

Hippocampus tissue of the brain is generally used to determine the neurotoxic effects of drugs in experimental studies. Hippocampus has two histologically different regions. Gyrus dentate (GD) region has granular cells, whereas cornuammonis (CA) region has pyramidal cells. Granular cells in GD region are the source of pyramidal cells in CA region, and resulting cells migrate toward CA region.[6-8] The aim of the present study was to determine the neurotoxic effect of repeated Δ – 9 – tetrahydrocannabiol administration on brain tissue and if neurotoxic effect was present on 5th day, whether this effect continued 16 days later.

MATERIALS AND METHODS

Animals

This study was conducted on 60 albino rats, regardless sex, with different ages and weighted 250-300 grams. Albino rats were housed in stainless-steel cages with wired cage lid in normal atmosphere. Liberal quantities of tap water and Commercial

rodent chow were provided ad libitum. Commercial rodent chow (HARLAN TEKIAD laboratory diets) has a caloric distribution of (62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt mixture), imported from Harlan Laboratories, Inc. company (Address: 8520 Allison Pointe Blvd., Suite 400, Indianapolis, Indiana 46520, USA).

Drugs: THC (Dronabinol) was purchased from THC Pharm GmbH (Frankfurt, Germany) and dispersed in a vehicle of ethanol, cremophor (Sigma-Aldrich, Madrid, Spain) and saline (1:1:18) (Llorente et al., 2007), animals received intraperitoneal injections of either THC (10 mg/kg) or vehicle according to the group tested.[9]

Groups: The animals were divided randomly into control and experimental groups.

Group I: Control group (20 healthy rats were used). Given 0.9% saline solution as intraperitoneal injections for 5 days, 4 times a day at 08.00, 12.00, 16.00 and 20.00 h using an insulin syringe. Sacrificed on 5th day at 20.00 after the last dose application.

Group II: Experimental group (20 healthy rats were used). Given 10 mg/kg Δ – 9 – tetrahydrocannabiol solution as intraperitoneal injections for 5 days, 4 times a day at 08.00, 12.00, 16.00 and 20.00 h. Sacrificed on 5th day at 20.00 after the last dose application.

Group III: Experimental group (20 healthy rats were used). Given 10 mg/kg Δ – 9 – tetrahydrocannabiol solution as intraperitoneal injections for 5 days, 4 times a day at 08.00, 12.00, 16.00 and 20.00 h. Sacrificed on 21st day, 16 days after the last dose.

At end of studies, rats were fasted overnight, anesthetized by intra-peritoneal injection with pentobarbital sodium (50 mg per kg body weight). After their death, rats were decapitated. Brain tissue was removed and placed in 10% formalin solution and embedded in paraffin for routine histological analyses. Five and 20 micron dissections were made from paraffin embedded tissues. Dissections were randomly sampled and stained with cresylic violet dye for histological examinations of hippocampus regions under a microscope.

Total number of neurons was determined by counting the number of neural cells in the hippocampus region using optic fractionation. Data obtained were evaluated using appropriate statistical methods (IBM SPSS Statistics 19, SPSS Inc., an IBM Co., Somers, NY, USA). Comparisons among the groups were performed using one-way analysis of variance. Two-way comparisons, on the other hand, were performed using Tukey's test, a post-hoc analysis. Kolmogorov-Smirnov test was applied to check whether the variables had a normal distribution.

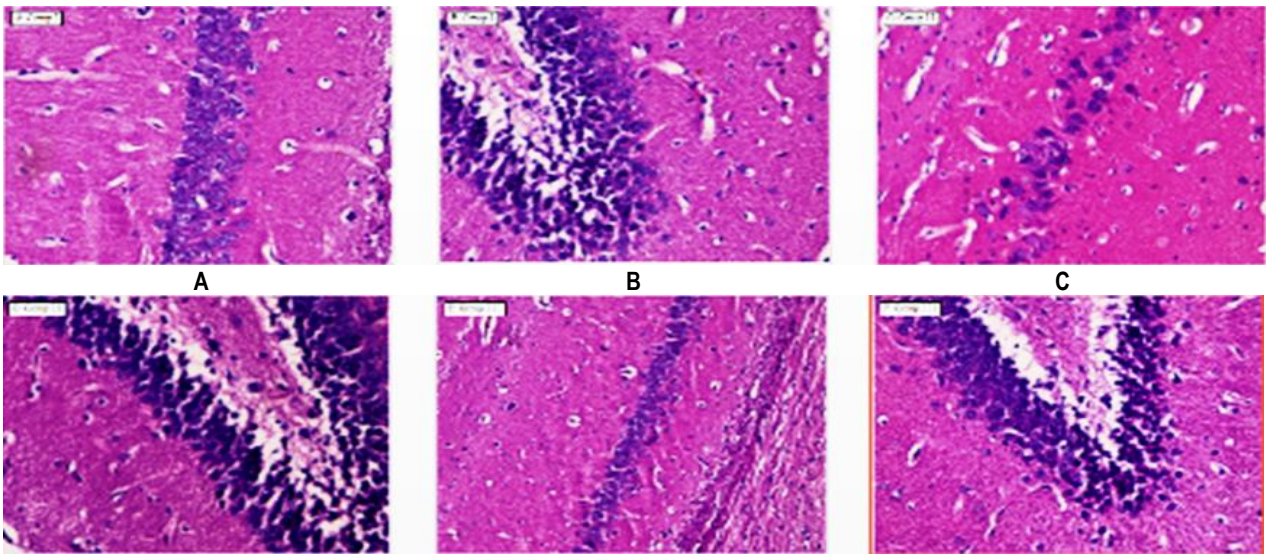

Figure 1 (A,B,C,D,E,F): Microscopic view (×400) of cornu ammonis and gyrus dentatus regions of rat hippocampus. A Photomicrograph of a section in the hippocampus of a group I. (A,B) hippocampus showing 5-6 compact layers of pyramidal cells in conus ammonis with vesicular nuclei. Stratum moleculare shows many glial cells among neuronal processes, and scattered nerve cells. Glial cells can be noticed near blood capillaries in gyrus dentatus. In group II (C, D) cells are scattered with loss of normal arrangement, some cell nuclei are deeply stained. In group III (E, F) cell regain its alignment and nuclei regain its vesicular appearance.

Table 1: Comparison of total number of neurons in CA and GD regions of hippocampus

Groups (n=5)	Total number of neurones [1] [mean±SE]	cv	Total number of neurones [2] [mean±SE]	cv	p
Group I	649864.4±21071.95	0.03	919195.40±82800.85	0.08	<0.001
Group II	244836.79±9388.26	0.03	458532.57±27649.69	0.05	<0.001
Group III	427350.00±26598.33	0.06	607762.80±25071.84	0.04	<0.001

P value is valid for within and between groups in CA and GD regions. SE: standard error, CV: coefficient of variation, CA: conus ammonis, GD: gyrus dentatus, 1: CA region, 2: GD region

RESULTS

Microscopic views of cells in CA and GD hippocampal regions of rats in study groups are given in Figure 1. Average numbers of neurons in hippocampal CA regions of study groups were compared. There were 62.33% less neurons in Group II than in Group I (control) ($P < 0.001$). Similarly, the average number of neurons in Group III was 34.25% less than the average of Group I ($P < 0.001$, Table 1). When Groups III and II were compared, conus ammonis (CA) region of rats in Group III had 74.54% more neurons than those of the ones in Group II ($P < 0.01$).

In terms of neuron numbers in gyrus dentatus(GD) region of hippocampus of rats in different study groups, there was a 50.12% decrease in Group II compared to Group I. Neuron number was 33.88% less in Group III than in Group I ($P< 0.001$) [Table 1]. When cell numbers in CA regions of rats in Groups II and III were compared, Group III had significantly higher numbers (28.08%) than Group II ($P< 0.01$). Group III had 16.24% more cells in GD region compared to Group II ($P< 0.001$).

DISCUSSION

The present study showed that repeated Δ THC doses resulted in decreases in neuron cells of rat hippocampus. There are some hypotheses about the mechanism by which Δ THC causes neurotoxicity in brain tissue. Δ9-THC causes destabilization of the lysosomal membrane via activation of the CB1 receptor and so, lysosomal branch of the apoptotic programmer induced by Δ9-THC in neurons.[10] Lysosomes are rising as vital controllers of the cell death. Originally thought to be stable organelles, only becoming destabilized during the end stages of cell death. [11]

Lysosomal destabilization occurred rapidly following exposure to Δ9-THC. Furthermore, the destabilization of lysosomes observed at 15 min occurs prior to the onset of activation of caspase-3.[12]

Data provide evidence which supports the hypothesis that the neonatal rat brain is more vulnerable to the neurotoxic influence of Δ(9)-tetrahydrocannabinol, suggesting that the cognitive deficits that are observed in humans exposed to marijuana during gestation may be due, in part, to abnormal engagement of the apoptotic cascade during brain development.[13]

Selective regions (CA2) of the hippocampus show persistent reductions in CB1 (cannabinoid 1) receptor expression and that these reductions are more widespread in female compared to male adolescents.[14] spatial learning impairment by Δ (9)-tetrahydrocannabinol (THC) in adolescent Sprague-Dawley rats.

We administered repeated doses of Δ THC to rats and found significant decreases in neuron numbers in CA and GD hippocampal regions of rats in treatment groups compared to the control group. In Group III, which had rats studied 16 days after Δ THC application, the average number of cells was statistically lower than the control group but higher than Group II, which had rats studied right after Δ THC application. This difference was thought to be caused by new cells produced during the 16 days from the last Δ THC application to the time when rats were sacrificed.

The hippocampus is one of only a few brain regions where production of neurons occurs throughout the lifetime of animals, including humans.[15]

The difference between Groups II and III for CA region was higher than the one for GD region. This difference reflected the cell production in GD region. In addition, when the effect of Δ THC application was studied 16 days after the administration (Group III), cell number was higher than Group II. We hypothesize that this finding could be due to the migration of newly produced cells in GD region to CA region of the hippocampus.

Neurogenesis persists in two adult brain regions: the ventricular subependyma and the subgranular cell layer in the hippocampal dentate gyrus (DG). Previous work in many laboratories has shown explicitly that multipotential, self-renewing stem cells in the subependyma are the source of newly generated migrating neurons that traverse the rostral migratory stream and incorporate into the olfactory bulb as interneurons. These stem cells have been specifically isolated from the subependyma, and their properties of self-renewal and multipotentiality have been demonstrated *in vitro*. In contrast, it is a widely-held assumption that the "hippocampal" stem cells that can be isolated *in vitro* from adult hippocampus reside in the neurogenic subgranular layer and represent the source of new granule cell neurons, but this has never been tested directly.

Primary cell isolates derived from the precise microdissection of adult rodent neurogenic regions were compared using two very different commonly used culture methods: a clonal colony-forming (neurosphere) assay and a monolayer culture system. Importantly, both culture methods generated the same conclusion: stem cells can be isolated from hippocampus-adjacent regions of subependyma, but the adult DG proper does not contain a population of resident neural stem cells. Indeed, although the lateral ventricle and other ventricular subependymal regions directly adjacent to the hippocampus contain neural stem cells that exhibit long-term self-renewal and multipotentiality, separate neuronal and glial progenitors with limited self-renewal capacity are present in the adult DG, suggesting that neuron-specific progenitors and not multipotential stem cells are the source of newly generated DG neurons throughout adulthood.[16]

CONCLUSION

Repeated doses of Δ THC were shown to cause decreases in rat hippocampus cells in the present study. In addition, it was also found that cell numbers increased after the Δ THC administration was stopped.

REFERENCES

1. Williams, G. D. Hash-oil manufacture: an important factor in the occurrence of adult burns in Jamaica. West Indian Med J, 1988; 37, 210-4.

2. Montgomery, L. Marijuana and tobacco use and co-use among African Americans: Results from the 2013, National Survey on Drug Use and Health. Addict Behav, 2015; 51, 18-23.

3. Gebler, S. Health effects of marijuana. J Psychosoc Nurs Ment Health Serv, 2015; 53, 16-7.

4. Molina-Holgado, F., González, M. I. & Leret, M. L. Effect of Δ9-tetrahydrocannabinol on short-term memory in the rat. Physiology & Behavior, 1995; 57, 177-179.

5. Li, X. J., Zhang, Y., Xu, C. Y., Li, S., Du, A. L., Zhang, L. B. & Zhang, R. L. Effects of polydatin on learning and memory and Cdk5 kinase activity in the hippocampus of rats with chronic alcoholism]. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2015; 31, 117-20.

6. Bartsch, T. & Wulff, P. The hippocampus in aging and disease: from plasticity to vulnerability. Neuroscience. 2015; 54, 174-178.

7. Michalovicz, L. T., Lally, B. & Konat, G. W. Peripheral challenge with a viral mimic upregulates expression of the complement genes in the hippocampus. J Neuroimmunol, 2015; 285, 137-42.
8. Phunchago, N., Wattanathorn, J. & Chaisiwamongkol, K. Tiliacora triandra, an Anti-Intoxication Plant, Improves Memory Impairment, Neurodegeneration, Cholinergic Function, and Oxidative Stress in Hippocampus of Ethanol Dependence Rats. Oxid Med Cell Longev, 2015; 918426.
9. Rubino, T., Vigano, D., Realini, N., Guidali, C., Braida, D., Capurro, V., Castiglioni, C., Cherubino, F., Romualdi, P., Candeletti, S., Sala, M. & Parolaro, D. Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuro-psycho-pharmacology, 2008; 33, 2760-71.
10. Gowran, A. & Campbell, V. A. A role for p53 in the regulation of lysosomal permeability by Δ9-tetrahydrocannabinol in rat cortical neurones: implications for neurodegeneration. Journal of Neurochemistry, 2008; 105, 1513-1524.
11. Brunk, U. T., Neuzil, J. & Eaton, J. W. Lysosomal involvement in apoptosis. Redox Rep, 2001; 6, 91-7.
12. Li, W., Yuan, X., Nordgren, G., Dalen, H., Dubowchik, G. M., Firestone, R. A. & Brunk, U. T. Induction of cell death by the lysosomotropic detergent MSDH. FEBS Lett, 2000; 470, 35-9.
13. Downer, E. J., Gowran, A. & Campbell, V. A. A comparison of the apoptotic effect of Delta (9) - tetrahydrocannabinol in the neonatal and adult rat cerebral cortex. Brain Res, 2007; 1175, 39-47.
14. Silva, L., Harte-Hargrove, L., Izenwasser, S., Frank, A., Wade, D. & Dow-Edwards, D. Sex-specific alterations in hippocampal cannabinoid 1 receptor expression following adolescent delta-9-tetrahydrocannabinol treatment in the rat. Neurosci Lett, 2015; 602, 89-94.
15. Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A. & Gage, F. H. Neurogenesis in the adult human hippocampus. Nat Med, 1998; 4, 1313-7.
16. Seaberg, R. M. & Van Der Kooy, D. Adult Rodent Neurogenic Regions: The Ventricular Subependyma Contains Neural Stem Cells, But the Dentate Gyrus Contains Restricted Progenitors. The Journal of Neuroscience, 2002; 22, 1784-1793.

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