Cannabis sativa and the Expression of Glial Fibrillary Acidic Protein (GFAP) in the Rat Hippocampal Astrocytes

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Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In rodents, the hippocampus has been studied extensively as part of a brain system responsible for spatial memory and navigation. The hippocampus is one of the first regions of the brain to suffer damage in Alzheimer’s disease. The hippocampus is one of the areas of the brain richly endowed with cannabinoid-1 (CB1), receptors. Recent studies in animal models and in the clinic suggest that CB1 receptor antagonists could prove useful in the treatment of parkinsonian symptoms. Delta-9-tetrahydrocannabinol, Δ9 – THC, activates dopaminergic neurons of the midbrain ventral tegmental area. Research had also reported a correlation between Cannabis use and increased cognitive function in schizophrenic patients. Advanced grades of Huntington’s

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disease, HD, showed an almost total loss of CB1 receptors in HD. The research article aims to investigate and compare the role of different preparation of *Cannabis sativa* via different routes of administration on learning, memory, and on the histology of the hippocampus in order to elucidate the best form of therapeutic use of the opiate in front of different type of diseases.

**Place and Duration of Study:** Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, between 2012 and 2014.

**Methodology:** The rats used for the study were divided into 5 groups comprising of A, B, C, D and E. Group A, 5 in number, served as the control and were given normal saline. Group B was divided into 3 subgroups, namely B1, B2 and B3, each comprising of 5 rats, and were given 0.41 mg/kg, 0.2 mg/kg, and 0.13 mg/kg, respectively, of Soxhlet extract of *Cannabis sativa* via oral ingestion. Group C comprised of subgroups C1, C2 and C3, each also made up of 5 rats was given 4grams, 2grams and 1grams, respectively, of grounded dried leaves of *Cannabis sativa* via inhalation. Group D also has 5 rats in each subgroup namely D1, D2, D3, and were given a mixture of 8grams, 4grams and 2 grams of grounded dried leaves of *Cannabis sativa* and 90 grams of animal feed in each case, while rats in Group E were given 1gm of 10% Tween 80 via oral ingestion. Before and after 28 days of administration of *Cannabis sativa*, the Morris water maze experiments on neurobehavioral were carried out. Sections were stained using GFAP immunostaining method. Data were expressed as means ± standard error of the mean and subjected to one way analysis of variance (ANOVA) using PRIMER, EXE, version 3.01. Significant differences between means were assessed by Student-Newman-Keuls post hoc test. 95% level of significance (*P* = .05) was used for the statistical analysis; while Microsoft excel 2010 package was used for graphs and error bars.

**Results:** *Cannabis sativa* obtained via soxhlet extraction technique has a mild and tolerable psychoactive effect compared to other preparations of *Cannabis sativa*.

**Conclusion:** This mode of preparation can be employed in the development of therapeutic strategies in view of its very low toxicity, thus presents a better method to be adopted in preparation of medical *Cannabis*.

**Keywords:** *Cannabis sativa*; hippocampus; astrocytes; albino rats; GFAP.

### 1. INTRODUCTION

Recent studies in animal models and in the clinic suggest that cannabinoid-1, (CB1), receptor antagonists could prove useful in the treatment of both Parkinsonian symptoms and levodopa-induced dyskinesia, whereas CB1 receptor agonists could have value in reducing levodopa-induced dyskinesia [1]. In another clinical trial, the role of CB1 receptors in schizophrenia was studied by administration of CB1 antagonist to patients. The group receiving the CB1 antagonist did not differ from the group receiving placebo on any outcome measure. It was found to be a safe and well-tolerated alternative treatment for schizophrenia [2]. Advanced grades of Huntington's disease, HD, showed an almost total loss of CB1 receptors [3] loss of cannabinoid receptors is also seen in the substantia nigra in HD. These findings suggest a possible therapeutic role of cannabinoid agonists in HD [4].

The major psychoactive compound in *Cannabis sativa* is Delta-9-tetrahydrocannabinol, Δ9 - THC, [5]. Δ9 -THC and related chemicals, known as cannabinoids, produce their psychoactive effects by acting at the CB1 receptor in brain areas associated with learning and memory and elsewhere [6]. A higher density of these proteins exists in brain areas that are critical for learning, memory, pain perception and reward processing. In fact, scientists think the activation of CB1 receptors in the brain’s reward pathways may underlie *Cannabis*’ likelihood for abuse [7,8]. Receptors for Δ9 -THC are present in the brain, especially in the limbic regions [9]. A 2008 study by the University of Melbourne of 15 heavy *Cannabis sativa* users and 16 controls found an average size difference for the smokers in the hippocampus (12 percent smaller) and the amygdala (7 percent smaller) [9]. The effects on the hippocampus probably account for acute impairment of short-term memory [9].

The CB1 receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum [10]. In the adult hippocampus intense signals for CB1 were observed as short woven fibers distributed in each layer of Ammon’s horn and dentate gyrus. CB1 also was detected in the innermost zone of the dentate gyrus molecular layer [10].
The hippocampus belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. Humans and other mammals have two hippocampi, one in each side of the brain. The hippocampus is located under the cerebral cortex; and in primates it is located in the medial temporal lobe, underneath the cortical surface. It contains two main interlocking parts: Ammon's horn and the dentate gyrus. [11,12]. Damage to the hippocampus can result from oxygen starvation (hypoxia), encephalitis, or medial temporal lobe epilepsy. People with extensive, bilateral hippocampal damage may experience anterograde amnesia—the inability to form or retain new memories [11]. A 2012 study [13] of the effects of marijuana on short term memories found that THC activates CB1 receptors of astrocytes. Acute Cannabinoids Impair Working Memory through Astroglial CB1 Receptor Modulation of Hippocampal [13]. In the hippocampus, astrocytes suppress synaptic transmission by releasing ATP, which is hydrolyzed by ectonucleotidases to yield adenosine [14].

Astrocytes are star cells that have multiple radially arranged cytoplasmic processes that can be appreciated only with special stains. Astrocytes are involved in information processing. Stimulation of astrocytes by neurotransmitters induces cell signaling to other astrocytes over relatively long distances [15] Astrocytes cytoplasm contains intermediate filaments composed of a distinct protein, glial fibrillary acidic protein (GFAP). Antibodies against this protein are routinely used to demonstrate reactive and neoplastic astrocytes. Historically, GFAP was the first immunostain to be used. Dimitri etc. http://neuropathology-

Astrogliosis is found to be a result of mechanical trauma, AIDS, dementia, prion infection and inflammatory demyelination diseases, and is accompanied by an increase in GFAP expression. GFAP is an immunohistochemical marker for localizing benign astrocyte and neoplastic cells of glial origin in the central nervous system [16]. GFAP increases at the periphery of ischemic lesion after neurodegenerative insults [17]. It is reported that in various neuroinflammatory diseases, the increased GFAP expression corresponds to the severity of astrogial activation [18]. However, the mechanism by which astrogial expression of GFAP is increased in neurodegenerative CNS remains unclear [19].

2. MATERIALS AND METHODS

Cannabis sativa was legally obtained from the national drug law enforcement agency (NDLEA), Akwa Ibom State command, and identified as such by the same agency with a reference code (an equivalent of voucher specimen number) NDLEA/AKSC/59/VOL.1/57.

The dried leaves of Cannabis sativa were grounded and weighed. The extract was obtained via soxlet extraction technique. [20]. Soxhlet extraction technique is a process which involves steam boiling of a substance, in this case Cannabis sativa, in an extraction flask connected to a steam bath via a connecting glass tubing. After being ground, Cannabis sativa were scooped into an extraction flask whose inlet and outlet were plugged with cotton wool. The cotton wool functioned to prevent particles of Cannabis sativa from escaping simultaneously with its vapor into the condenser. Following the introduction of ethanoic acid into the flask containing Cannabis sativa, the source of electric current was switched to ON position. This automatically supplied alternating current that powered the steam bath. As the name indicated, anti-bumping agents / granules introduced into the flaks functioned to prevent bumping. Clamp and stand were used to hold the extraction flask to the desired position. Vaseline jelly rubbed at the edges where the flask and glass tubing came in contact with a lid made such contact airtight to prevent escape of vapor. The emerging vapor was channeled to an inverted U-shaped condenser. Any stream of vapor that managed, by virtue of its velocity, to reach the apex of the inverted U-shaped condenser, subsequently crossed over to the other arm of the condenser parallel to the condenser. This particular stream of vapor on condensation constituted the extract of Cannabis sativa, while vapor devoid of adequate velocity fell back and repeated the procedure till it finally passed over.

The extract was screened for the presence of alkaloids, saponins, tannins, and cardiac glycoside using standard methods [21,22].

The aim of Ld50 study was to ascertain the highest dose of cannabis sativa at which the mice/rats will survive and the lowest dose at which the mice/rats will die; hence, the dosage the mice/rats will tolerate. In toxicology, LD50 (abbreviation for "lethal dose, 50\%"), is the dose required to kill half the members of a tested population after a specified test duration. Ld50
figures are frequently used as a general indicator of a substance's acute toxicity. The test was created by Trevan in 1927. In this study, mice were used for the LD$_{50}$ though rats were the animals used for the research proper. This is due to inability of mice to withstand/tolerate toxicity like rats, hence, it is assumed, that whatever amount in grams of toxicity of any substance mice was able to tolerate, rats will no doubt withstand and tolerate such. Based on this assumption, this idea is a sort of precaution to forestall death of experimental rats in research.

The route of administration was oral ingestion, using insulin syringe fixed to a metal oropharyngeal cannula. Lorke's method was used for the determination of LD$_{50}$. A total of thirty six adult male mice was used for the LD$_{50}$ study/research. Based on the outcome of this study, the maximum dosage of the active ingredients of Cannabis sativa that produced 100% mortality was 700 mg/kg, while the minimum dosage that produced 0% mortality was 600 mg/kg.

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\text{LD}_{50} = \sqrt{AB} = \sqrt{600 \times 700} = 648.07 \text{ mg/kg}
\]

For the administration of Cannabis sativa to mice, 10%, 20% and 30% of $\sqrt{600 \times 700}$ (648.07 mg/kg) was used. Where:

- The value of 10% of 648.07 mg/kg = low dose = 64.81 mg/kg
- The value of 20% of 648.07 mg/kg = middle dose = 129.61 mg/kg
- The value of 30% of 648.07 mg/kg = high dose = 194.42 mg/kg

2.1 Rats and Treatment

A total of fifty (55) adult male albino Wistar rats were used for the research. The rats were divided into five groups: A, B, C, D, and E.

Group A, the control, was made up of five male rats and were given normal saline.

Group B, was given the extract of Cannabis sativa via oral ingestion, using insulin syringe fixed to a metal oropharyngeal cannula. The extract was immiscible with water, and was made miscible by mixing with 10% Tween 80. The 10% Tween 80 was prepared by adding 10 mls of Tween 80 to 90 mls of distilled water. Following the preparation of the stock solution, insulin syringe was used to measure and administer the exact quantity needed on each occasion on the rats. Group B, comprised of three subgroups, namely, B1, B2 and B3.

B1 comprising of 5 male rats was given 0.41 mg/kg body weight of Cannabis sativa via oral ingestion.

B2 comprising of 5 male rats was given 0.2 mg/kg body weight of Cannabis sativa via oral ingestion.

B3 comprising of 5 male rats was given 0.13 mg/kg body weight of Cannabis sativa via oral ingestion.

Group C, was exposed to smoke from ground dried leaves of Cannabis Sativa. Group C comprised of three subgroups, namely, C1, C2, and C3. This was done by placing red-hot charcoal at the bottom level of a desiccator. The required number of grams of dried grounded Cannabis sativa leaves was on each occasion spread on top of the red-hot charcoal. Galvanized wire gauze was immediately placed above this compartment to separate it from the compartment above it containing the rat and to serve as a platform for the rat. The desiccator was covered with its lid once the rat was placed inside, and timing started. The rats were allowed to inhale the smoke for three minutes. After every three minutes, the lid was opened for ten seconds to alternate the flow of smoke and fresh air to the animals to mimic puffing.

C1 comprised of 5 male rats. All the rats in this group were exposed to smoke from 4grams of ground dried leaves of Cannabis sativa for three minutes; with 10 seconds interval between each minute to enable them breathe in fresh air.

C2 comprised of 5 male rats. All the rats in this group were exposed to smoke from 2 grams of ground dried leaves of Cannabis sativa for three minutes; with 10 seconds interval between each minute to enable them breathe in fresh air.

C3 comprised of 5 male rats. All the rats in this group were exposed to smoke from 1gram of ground dried leaves of Cannabis sativa for three minutes; with 10 seconds interval between each minute to enable them breathe in fresh air.

Group D, was given a mixture of ground dried leaves of Cannabis sativa and animal feed.
Group D, comprised of three subgroups, namely, D1, D2, and D3.

D1 comprising of 5 male rats were given a mixture 8 grams of ground dried leaves of *Cannabis sativa* and 90 grams of animal feed.

D2 comprising of 5 male rats were given a mixture 4 grams of ground dried leaves of *Cannabis sativa* and 90 grams of animal feed.

D3 comprising of 5 male rats were given a mixture 2 grams of ground dried leaves of *Cannabis sativa* and 90 grams of animal feed.

Group E, has only one group, E. It comprised of 5 male rats. They were given 1 gm of 10% Tween 80 via oral ingestion using insulin syringe fixed to a metal oropharyngeal cannula.

Behavioral studies on the water maze were carried out on the rats before and after administration of *Cannabis sativa*. The Morris water maze is one of the most widely used tasks in behavioral neuroscience for studying the physiological processes and neural mechanisms of spatial learning and memory. The apparatus consisted of a large circular pool (tank) of about 2.14 m in diameter x 0.40 m height. The interior/inside of the tank was painted white. The tank was filled with clean tap water. A platform of about 24 cm length and 9 cm in diameter was placed in one quadrant of the tank. This particular platform was used during testing session. Rats were placed on the circular pool of water so that they escape onto a hidden platform. The platform was made hidden first by making its topmost surface to be below the water surface, and secondly by making the water in the circular pool opaque; by adding and mixing a given quantity of powdered milk to the water. By so doing, the platform offered no local cues to guide escape behavior. Another platform of also 9 cm in diameter but of about 1 cm above water surface/level was used during training.

2.2 Training of the Rat for the Water Maze

Each rat underwent three consecutive trials. First, the rat was placed on the exposed platform for about 15 seconds for acclimatization. Inside the water tank, four start positions were noted, and the rat was taken to any of the four start position. A maximum time of 60 seconds was set aside for each rat to swim and locate the exposed platform at one of the start positions. The time taken for the rat to reach the platform was noted. However, if the rat was not able to reach/locate the exposed platform, it was guided to it. Whether the rat located the platform by itself or was guided to locate it, the rat was allowed to sit on the platform for about 15 seconds, after which it was dried and returned to a cage. This procedure was repeated for two more training but starting from a different starting point on each occasion while maintaining the same position for the platform.

2.3 Testing of the Rat for the Water Maze

In the testing each rat was made to undergo three trials per session, this was done for four sessions. Testing was done following the training session. At the beginning of each testing session, the rat was placed on the platform for about 10 seconds. The testing proper was done by placing the rat in water at any of the four points on the maze wall. The testing for each rat for each session came to an end once the rat located the platform and climbed onto it.

With the aid of a stopwatch, the time taken for each rat to climb onto the platform was noted/recorded, this time was referred to as the latency period. The average of the latency period was calculated from the values of the three latencies of a session, this indicated the performance of the animal. The rat was removed from the water maze and placed onto the platform on each occasion it was unable to locate the platform within 60 seconds.

The inter-trial period was the time interval between two testing/trial periods. The duration was 10 seconds. The rat was made to stay on the platform during this period before the new trial/testing. The rat was later placed in a cloth containing casing fitted with light to get it dried up and for warmth.

2.4 Probe Trial

The rat was allowed 60 seconds for the probe trial. During this 60 seconds period, the number of times the rat swam across the initial position of the platform was noted and recorded.

2.5 Visible Platform (Cued Water-maze Task)

The same procedure used in testing and probe trial was adopted, the only difference being that the platform was made visible, just as the name implies, and the time taken by the rat to locate the visible platform was noted.
Each rat was weighed weekly and observed for any change in behavior. The rats were anaesthetized by placing in a desiccator containing cotton wool soaked in chloroform. Each rat was gently placed inside the desiccator and covered with the lid for a few minutes to make the rats unconscious. The rats were perfused through the vascular system. The saline was ran first until the effluent fluid was clear. The fixative was then perfused until the whole body of the rat was hard and inflexible. The rat brains were dissected out from each group and taken to laboratory for histological, histochemical and immunohistochemical analysis. The analyses were done on the CA.

The tissues were processed for paraffin embedding following fixation. Drury and Wallington [23] recommended procedure was adopted. GFAP immunostaining was done using Avidin biotin Immunoperoxidase method.

Data were expressed as means ± standard error of the mean (M±SEM) and subjected to one way analysis of variance (ANOVA) using PRIMER, EXE, version 3.01. Significant difference between means was assessed by Student-Newman-Keuls post hoc test. 95% level of significance (P = .05) was used for the statistical analysis, while Microsoft excel 2010 package was used for graphs and error bars. Digital USB camera, scope photo 1.5 MP was connected to a light microscope and was used in assessing the slides of hippocampal tissues following various staining methods. It was also used to study the intensity of GFAP expression without cell counting.

3. RESULTS

In Table 1 and Fig. 1, mean values for the behavior of rats on Morris water maze during memory retention (retrieval) test before administration of *Cannabis sativa* among the various groups were compared and analyzed using ANOVA. Each data point represents the average performance of each subject across four sessions. The latencies to target significantly decreased across trials. From this one can deduce that the rats in all the groups, both the control and the experimental got used to the exposed escape platform location with time as the trial continued, hence, the gradual decrease in the time along the path length and time spent in locating the target across training sessions. Moreover, there was no specific trend of differences in latencies across sessions in a group compared to sessions in other groups.

| Group | Subgroups | Session 1 F=0.65 P=0.763 | Session 2 F=1.10 P=0.383 | Session 3 F=4.48 P=0.000 | Session 4 F=3.89 P=0.000 |
|-------|-----------|--------------------------|--------------------------|--------------------------|--------------------------|
| A     | A         | 15.40±0.51               | 13.20±1.74               | 6.40±0.60                | 3.00±0.45                |
| B     | B1        | 15.20±0.58               | 14.00±0.55               | 12.00±0.32*              | 9.40±0.68*               |
|       | B2        | 14.20±0.80               | 12.20±0.66               | 10.40±0.15*              | 8.60±0.75*               |
|       | B3        | 15.00±0.45               | 11.40±0.60               | 9.80±0.58                | 8.00±0.71*               |
| C     | C1        | 14.60±0.51               | 12.20±0.58               | 9.80±0.58*               | 7.00±1.22*               |
|       | C2        | 14.40±0.51               | 11.20±0.58               | 9.80±0.37*               | 6.60±1.17*               |
|       | C3        | 14.80±0.37               | 11.60±0.68               | 10.40±0.75*              | 7.60±1.03*               |
| D     | D1        | 14.60±0.60               | 12.20±0.37               | 10.40±0.40*              | 9.30±0.40*               |
|       | D2        | 13.60±0.75               | 11.40±0.75               | 9.80±0.92*               | 6.40±0.81*               |
|       | D3        | 14.80±0.97               | 11.80±0.80               | 9.20±1.07*               | 7.00±0.89*               |
| E     | E         | 14.80±0.37               | 11.40±0.68               | 9.80±0.37*               | 6.20±0.37                |

Key: Values are expressed as mean±standard error of mean (M±SEM), *= significant compared to control at P = .05, A = control group

B1 = rats given 0.41 mg/kg body weight of soxhlet extract of *C. s.* via oral ingestion, B2 = rats given 0.20 mg/kg body weight of soxhlet extract of *C. s.* via oral ingestion, B3 = rats given 0.13 mg/kg body weight of soxhlet extract of *C. s.* via oral ingestion

C1 = rats exposed to smoke from 4 g of ground dried leaves of *C. s.*, C2 = rats exposed to smoke from 2 g of ground dried leaves of *C. s.*, C3 = rats exposed to smoke from 1 g of ground dried leaves of *C. s.*

D1 = rats given a mixture of 8 g ground dried leaves of *C. s.* & 90 g of animal feed, D2 = rats given a mixture of 4 g ground dried leaves of *C. s.* & 90 g of animal feed, D3 = rats given a mixture of 2 g ground dried leaves of *C. s.* & 90 g of animal feed, E = rats given 1 gm of 10% Tween 80 via oral ingestion
Both shorter and longer durations of time were observed across sessions in all the groups. Swim latency was higher in a session in some groups and shorter in a session of other groups. There were no significant differences in latencies of all the experimental groups compared to the control.

In Table 2 and Fig. 2, the path lengths and latencies to swim to the hidden platform during repeated acquisition (memory) retention test are shown. Rats in all the groups, both the control and the experimental, also exhibited significant decreases in path length and latency to find the hidden platform across the acquisition trials, signifying that they learned and retain in their memory the path and location of the formally exposed platform, now hidden. The path lengths were significantly reduced in sessions 2, 3, and 4, compared with session 1 within group. Likewise, the escape latencies were significantly decreased in sessions 2, 3, and 4, compared with trial 1 within group. Across the groups, escape latency was shorter in group B compared to other experimental groups. The escape latency in B1 was shorter compared to that of C1, and D1, that received high doses in their various regimen. The same trend was observed in B2 and B3, when compared with C2, C3, and D2, D3, respectively. There were no significant differences in swim speed across trials within and between groups.

For the probe trial, Fig. 3; after administration of Cannabis sativa, the rats were allowed 60 seconds, during which the number of times the rat swam across the initial position of the exposed platform, now hidden, was noted and recorded. The rats in group B swam across the initial position of the exposed platform, now hidden, more often than rats in other experimental groups, namely C, D, and E; the control group A included. In the subgroups within groups, like in B1, B2, B3; C1, C2, C3, and D1, D2, D3, the number of times the rats swam across or loiter around the target decreased in direct proportion to decrease in dosage in each case. Moreover, in all of these cases, the values are higher than that of control group A and experimental group E.

In Fig. 4, the time taken the rats to swim across and locate the exposed platform was shorter in the experimental group B (B1, B2, B3) compared to other experimental groups, C1, C2, C3, and D1, D2, D3. However, rats in B1, B2 and B3 given high, middle and low doses of the extract.
of *Cannabis sativa* spent more time in locating the target compared to the time spent to locate the target by the control group A and experimental group E.

**Table 2. Comparison of latencies of rats in Morris water maze during memory retention (retrieval) test after administration of *Cannabis sativa***

| Groups | Sub groups | Latencies |          |          |          |          |
|--------|------------|-----------|----------|----------|----------|----------|
|        |            | Session 1 | Session 2 | Session 3 | Session 4 |
|        |            | F=2.78    | F=2.79   | F=4.78   | F=5.82   |
| A      | A          | 13.40±0.51| 11.00±0.45| 9.20±0.37| 6.80±0.37|
| B      | B1         | 15.40±1.03| 14.40±0.87| 12.60±0.81*| 11.00±0.23*|
|        | B2         | 16.00±0.32| 12.40±0.68| 11.60±0.75| 10.40±0.51*|
|        | B3         | 14.80±0.37| 12.60±0.75| 11.60±0.75| 10.00±0.49*|
| C      | C1         | 16.60±0.51*| 14.60±0.81*| 13.60±0.24*| 11.80±0.80*|
|        | C2         | 15.60±0.51| 14.20±0.58| 13.20±0.58*| 11.40±0.87*|
|        | C3         | 15.00±0.55| 13.00±0.71| 11.40±0.60| 10.00±0.63*|
| D      | D1         | 16.20±0.66*| 14.80±0.86*| 14.00±0.63*| 11.60±0.81*|
|        | D2         | 16.00±0.55| 13.20±1.02| 12.00±0.89*| 9.60±0.51*|
|        | D3         | 15.00±0.45| 12.00±0.71| 11.40±0.68| 10.60±0.40*|
| E      | E          | 12.50±0.55| 11.30±0.71| 9.80±0.45*| 7.00±0.45*|

**Key:** Values are expressed as mean±standard error of mean (S±SEM), * = significant compared to control at *P* =.05

**Fig. 2.** Comparison of latencies of rats in Morris water maze during memory retention (retrieval) test after administration of *Cannabis sativa*

Key: NS = not significant compared to control at *P* =.05, * = significant compared to control at *P* =.05
Fig. 3. Comparison of results of probe trial of rats in Morris water maze during memory retention (retrieval) test after administration of Cannabis sativa

Key: NS = not significant compared to control at P = .05

Fig. 4. Comparison of results of cued task (visible platform) of rats in Morris water maze during memory retention (retrieval) test after administration of Cannabis sativa

Key: * = significant compared to control at P = .05

The plates, A to K, below portrayed the effect of different treatments over GFAP expression. The effects are described individually for each plate in the notes immediately after the plates. Moreover, in this study, increased GFAP expression were observed only on those reactive and hypertrophied astrocytes, irrespective of whether these reactive astrocytes are numerous or scarcely populated in their location. However, normal astrocytes showed slight GFAP expression wherever they are located irrespective of their number in such locations.
Plate A. Photomicrograph of cross section of the hippocampus of albino rat given distilled water. Group A (control). Immunostaining method for GFAP. X400. Inference: normal-appearing astrocytes (ast). slightly positive. normal GFAP expression on astrocytes.

Plate B. Photomicrograph of cross section of the hippocampus of albino rat given 0.41 mg/kg of soxtlet extract of Cannabis sativa via oral ingestion. Group B1. Immunostaining method for GFAP. X400. Section is characterized by a few reactive astrocytes (R-ast), (red arrows). Inference: strongly positive, that is increased GFAP expression but only on a few reactive astrocytes.

Plate C. Photomicrograph of cross section of the hippocampus of albino rat given 0.20 mg/kg of soxtlet extract of Cannabis sativa via oral ingestion. Group B2. Immunostaining method for GFAP. X400. section is characterized by reactive astrocytes (R-ast) and normal astrocytes (ast). (red arrows). inference: moderately positive. moderate GFAP expression on the observed reactive astrocytes.

Plate D. Photomicrograph of cross section of the hippocampus of albino rat given 0.13 mg/kg of soxtlet extract of Cannabis sativa via oral ingestion. Group B3. Immunostaining method for GFAP. X400. Section is characterized by increased GFAP expression on the reactive/hypertrophied astrocytes (R-ast) and slight expression on a few normal astrocytes (red arrows). Inference: slightly positive.

Plate E. Photomicrograph of cross section of the hippocampus of albino rat exposed to smoke from 4g of grounded dried leaves of Cannabis sativa. Group C1. Immunostaining method for GFAP. X400. section is characterized by increased GFAP expression on numerous reactive/hypertrophied astrocytes (R-ast) with perivascular processes (Pv.p) (red arrows). inference: strongly positive.

Plate F. Photomicrograph of cross section of the hippocampus of albino rat exposed to smoke from 2g of grounded dried leaves of Cannabis sativa. Group C2. Immunostaining method for GFAP. X400. Section is characterized by increased GFAP expression on reactive/hypertrophied astrocytes (R-ast) (red arrows). Inference: strongly positive.

Plate G. Photomicrograph of cross section of the hippocampus of albino rat exposed to smoke from 1g of grounded dried leaves of Cannabis sativa. Group C3. Immunostaining method for GFAP. X400. Section is characterized by increased GFAP expression on reactive/hypertrophied astrocytes (R-ast) and normal expression on a few normal astrocytes (ast) (red arrows). Inference: strongly positive.
Plate H. Photomicrograph of cross section of the hippocampus of albino rat given a mixture 8g of grounded dried leaves of Cannabis sativa and 90g of animal feed. Group D1. Immunostaining method for GFAP. X400. Section is characterized by increased GFAP expression on numerous reactive/hypertrophied astrocytes (R-ast) and thick perivascular processes (Pv-p) (red arrows). Inference: strongly positive.

Plate I. Photomicrograph of cross section of the hippocampus of rat given a mixture 4g of grounded dried leaves of Cannabis sativa and 90g of animal feed. Group D2. Immunostaining method for GFAP. X400. Section is characterized by increased GFAP expression on numerous reactive/hypertrophied astrocytes (R-ast). (red arrows). Inference: strongly positive.

Plate J. Photomicrograph of cross section of the hippocampus of rat given a mixture 2g of grounded dried leaves of Cannabis sativa and 90g of animal feed. Group D3. Immunostaining method for GFAP. X400. Section is sparsely populated by reactive astrocytes which are the only astrocytes where increased GFAP expression was observed. Inference: slightly positive.

Plate K. Photomicrograph of cross section of the hippocampus of rat given 1gm of 10% tween 80. Group E. Immunostaining method for GFAP. X400. Inference: normal GFAP expression in normal-appearing astrocytes (ast). slightly positive

4. DISCUSSION

In Table 1 and Fig. 1, the latencies to target significantly decreased across training trials. From this one can deduce that the rats in all the groups, both the control and the experimental got used to the exposed escape platform location with time as the trial continued, hence, the gradual decrease in the time along the path length and time spent in locating the target across trial sessions.

In Table 2 and Fig. 2, the path lengths were significantly reduced in sessions 2, 3, and 4, compared with session 1 within group. Likewise, the escape latencies were significantly decreased in sessions 2, 3, and 4, compared with trial 1 within group. Across the groups, escape latency was shorter in group B compared to other experimental groups. The escape latency in B1 was shorter compared to that of C1, and D1, that received high doses in their various regimen. The same trend was observed in B2 and B3, when compared with C2, C3, and D2, respectively. There were significant differences in swim speed across trials within and between groups. From this it can be deduced that Cannabis sativa dose-dependently disrupted the recall of the target. However, the memory disruption was not severe in Subgroups B1, B2, and B3 compared to other groups and their subgroups given different preparation of Cannabis sativa.

For the probe trial, Fig. 3; the rats in group B swim across the initial position of the exposed platform, now hidden, more often than rats in other experimental groups. In the subgroups within groups, like in B1, B2, B3; C1, C2, C3, and D1, D2, D3, the number of times the rats swim across or loiter around the target decreased in direct proportion to decrease in dosage in each case. Moreover, in all of these cases, the values are higher than that of control group A and experimental group E, indicating that Cannabis sativa affected the memory recalling capacity positively in some subgroups but negatively in some others. In subgroups C1, C2, C3, D1, D2, and D3, it can be deduced that there was memory disruption in these groups because the rats did not swim across the target more often; nothing pointed to the fact that they have bias to a target. However, it can be deduced that it is dose dependent, because the frequency of swimming across target decreased across trials in each subgroup.

The frequency of swimming across target by group B and its subgroups though more often than in other experimental groups, was also higher compared to the control group A and experimental group E. Based on the trend thus far, it is supposed to be the other way round; one possible reason for this might be attributable to memory stimulating effect of the extract. Perhaps, made the memory more active than in normal condition, hence, the ability of the rats in subgroups B1, B2, and B3, to have sharp bias to the target and swam as much as possible across its location than normal rats without Cannabis sativa.

The result of these findings is corroborated by the works of Russo, et al., [24] in which it was demonstrated that exposure to Cannabis sativa smoke 30 min before the retention test impaired the ability of mice to return to the location where the platform had been previously located; suggesting impaired retrieval of recently learned spatial information, independent of effects on
acquisition. Thus, two different aspects of cognition (acquisition and retrieval) were impaired by exposure to Cannabis sativa smoke.

In Fig. 4, the time taken the rats to swim across and locate the exposed platform was shorter in the experimental group B (B1, B2, B3) compared to other experimental groups, C1, C2, C3, and D1, D2, D3. However, rats in B1, B2 and B3 given high, middle and low doses of the extract of Cannabis sativa spent more time in locating the target compared to the time spent to locate the target by the control group A and experimental group E. These observation contradicts the insinuation in Fig. 3 above, about the ability of the rats in subgroups B1, B2, and B3, having sharp bias to the target and swam as much as possible across its location than normal rats without Cannabis sativa. Otherwise, in the same vein, rats in subgroups B1, B2, and B3 would had spent lesser time to locate the target than the control.

In another longitudinal study examining adolescents exposed to cannabis prenatally, current heavy marijuana users had lower scores on processing speed and immediate and delayed memory as compared to controls, even after accounting for performance prior to the onset of use. However, no differences were found between the users who had been abstinent at least three months and controls [25]. Thus, it is possible that, in adolescents with heavy marijuana use, neurocognitive deficits in the areas of attention, verbal learning/memory, and processing speed persist beyond one month of abstinence, but largely remit after three months of sustained abstinence.

Glial fibrillary acidic protein (GFAP), is considered a marker protein for astroglial activation [16]. It is reported that in various neuroinflammatory diseases, the increased GFAP expression corresponds to the severity of astrogial activation [16,18]. Though this mechanism is incompletely understood, in this study, increased GFAP expression was used to make certain deductions about reactive and hypertrophied astrocytes.

Iversen notes that, although an extensive literature describes lasting neurotoxic effects in chronic cannabis users, little scientific evidence supports these claims. In fact, delta-9-tetrahydrocannabinol, Δ 9 - THC (and other cannabinoids that do not act on cb1 receptors) have been reported to have neuroprotective effects, perhaps owing to their antioxidant properties [26].

Functional and structural variations in the hippocampus have been linked to reduced memory performance and psychotic symptoms [27]. Cannabis exposure produces reduced activation in the hippocampus during verbal and visual learning tasks [28,29]. The potential medical applications of cannabis in the treatment of painful muscle spasms and other symptoms of multiple sclerosis are currently being tested in clinical trials. Medicines based on drugs that enhance the function of endocannabinoids may offer novel therapeutic approaches in the future [30].

Pope and colleagues [31] examined neurocognition in chronic heavy Cannabis sativa users, importantly, after 28 days of monitored abstinence. No differences were seen between users and controls on a comprehensive neuropsychological battery on testing day 28. Conversely, a study by Bolla et al. [32] did find differences between heavy and light Cannabis sativa users after 28 days of abstinence, along with a dose-dependent relationship between joints smoked per week prior to enrollment and performance on measures of psychomotor speed, executive functioning, and manual dexterity. A meta-analysis by Grant and colleagues [33] attempted to identify the long-term effects of Cannabis sativa consumption on cognition, and reported small effects in the domains of learning, memory, and overall neurocognitive performance. Thus, subtle neurocognitive effects observed in heavy adult Cannabis sativa users may be attributable to “residual” sequelae present only in the first few hours or days of stopping Cannabis sativa use [33].

The use of Cannabis has increased dramatically in several countries over the past few decades, the rates of psychosis and schizophrenia have not generally increased [34]. Conversely, research from 2007 reported a correlation between Cannabis use and increased cognitive function in schizophrenic patients [35].

It has been suggested that such effects can be reversed with long term abstinence. However, the study indicated that they are unsure that the problems were caused by Cannabis sativa alone [36].
5. CONCLUSION

There is a wide spread impression that consumption of *Cannabis sativa* is harmful. Documented evidence showed that *Cannabis sativa* had been used for centuries as a medicinal plant, but was later considered illegal and withdrawn from clinical practice resulting from its abuse. The findings of this study thus suggested that the extract of *Cannabis sativa* obtained via soxhlet extraction technique has a mild and tolerable psychoactive effect compared to other preparations of cannabis sativa, thus presents a better method to be adopted in preparation of medical cannabis. In view of the very low toxicity and the generally benign side effects of these extract neglecting or denying their clinical potential is wrong. The therapeutic potential of cb1 agonists is huge.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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