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Repeated Acute Oral Exposure to *Cannabis sativa* Impaired Neurocognitive Behaviours and Cortico-hippocampal Architectonics in Wistar Rats

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Summary: The most abused illicit drug in both the developing and the developed world is Cannabis disposing users to varying forms of personality disorders. However, the effects of cannabis on cortico-hippocampal architecture and cognitive behaviours still remain elusive. The present study investigated the neuro-cognitive implications of oral cannabis use in rats. Eighteen adult Wistar rats were randomly grouped to three. Saline was administered to the control rats, cannabis (20 mg/kg) to the experimental group I, while Scopolamine (1 mg/kg. ip) was administered to the last group as a standard measure for the cannabis induced cognitive impairment. All treatments lasted for seven consecutive days. Open Field Test (OFT) was used to assess locomotor activities, Elevated Plus Maze (EPM) for anxiety-like behaviour, and Y maze paradigm for spatial memory and data subjected to ANOVA and T test respectively. Thereafter, rats were sacrificed and brains removed for histopathological studies. Cannabis significantly reduced rearing frequencies in the OFT and EPM, and increased freezing period in the OFT. It also reduced percentage alternation similar to scopolamine in the Y maze, and these effects were coupled with alterations in the cortico-hippocampal neuronal architectures. These results point to the detrimental impacts of cannabis on cortico-hippocampal neuronal architecture and morphology, and consequently cognitive deficits.

Keywords*:* Anxiety, Cannabis toxicity, Cortex, Memory, Hippocampus

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INTRODUCTION

Agents of abuse have been demonstrated to exert detrimental impact upon social, psychological and cognitive behaviour in individual users, thereby affecting their personality. In the recent epoch, the number of illicit drug consumption has unfortunately increased and concerns have been articulated on the dangers of these agents in various societies in the world.

Preparations from Cannabis, with common names like hashish, grass, weed and marijuana are the most consumed illicit drugs worldwide with one out of ten users developing daily use pattern (Rosales-Corral et al, 2015).

Although, cannabis is perceived by many to be less addictive compare to other drugs of abuse, such as cocaine, in reality, it causes more dependency (Bianconi et al, 2016), enhanced vulnerability to addiction and psychiatric disorders in users (Yasmin et al, 2014) and increased intake of other drugs of abuse (Schuster et al, 2015; Subbaraman and Kerr, 2015).

Convergence and replicated findings have associated the toxicity in cannabis use to varying complexes in psycho-cognitive impairments, such as learning and memory, mood, planning and other executive functions (Ashley et al, 2016; Hall, 2015; Desrosiers et al, 2015). It has also been linked to severe transient psychotic symptoms (Myles et al, 2015; Gage et al, 2015).

The major psychoactive constituent of cannabis, Tetrahydrocannabinol (THC) acts on the cannabinoid (CB) receptors that is widely expressed in the cortices, the basal ganglia, and the hippocampal formation, resulting in a wide topographical toxicity pattern across the descript regions of the brain (Downer and Campbell, 2010). However, the implication of cannabis on cortico-hippocampal neuronal architecture and spatial cognitive behaviours still remain inconclusively known.

Despite all the convincing neuropsychological effects of cannabis, cannabis and its products are becoming increasingly the most popular illicit

psychoactive substances used among teenagers; albeit, it is postulated to be relatively safe and the attributed medical benefits in epilepsy (Tzadok et al, 2016), pain (Lynch, 2016), cancer (Velasco et al, 2016) and inflammation (Juknat et al, 2016) have been established.

Therefore, investigating the associated toxicity in cannabis use has become an important public health issue, thus, we investigated the effects of repeated daily oral use of cannabis on locomotor activities, anxiety-like behaviours, cognitive indices and cortico-hippocampal neural architectonic in rats. It is apparent that, cannabis induced psychocognitive derailment is both social and economic burden, thus its outturn in this work will provide further awareness to these burdens.

MATERIALS AND METHODS

Preparation of *Cannabis sativa* Extract

Cannabis plant leaves were obtained as donation for research from the National Drug and Law Enforcement Agency (NDLEA) in Kwara State, Nigeria, dried, blended to powdery particles and weighed. The particles were soaked in distilled water and kept for about eighteen hours. The mixture was filtered, the filtrate was oven dried under 45^{o}C and the dried filtrate was weighed and stored in an air tight container until ready to use.

Drugs

The Scopolamine hydrobromide used in this study was purchased from Sigma (St. Loius, MO, USA). Scopolamine was dissolved in saline to make a final concentration of 1 mg/kg.bw, and was administered intraperitoneally (*i. p*).

Animal Care

Eighteen adult Wistar rats with average weight 200 ± 20 g at the time of acquisition and acclimatization were used in this study. The animals were housed in the Animal holding of the Faculty of Basic Medical Sciences, University of Ilorin, six in a cage with free access to water and food, under standard laboratory condition of 22 ± 2°C temperature and 12/12 h light-dark cycle.

Treatments schedule

The rats were randomly distributed into three (3) groups (n = 6) as follows:

1. Saline-control: received saline (1 ml/kg).
2. Experimental group: received Cannabis sativa (20 mg/kg/day p.o) (Omar et al, 2013; 2014)
3. Standard control: received scopolamine (1 mg/kg/day IP) (Imam et al, 2016[a])

All procedures were scheduled and carried out during the light phase between 9:00 and 15:00. All groups contain 6 rats each and treatments were for seven consecutive days.

Ethical approval

All experimental procedures were performed in accordance to NIH guidelines on use and care of laboratory animals. Ethical approval was received from the University of Ilorin ethics committee (UIL/COHS/FBMS/ET1025) and the National Drug and Law Enforcement Agency (NDLEA) in Kwara State, Nigeria, gave her consent to use cannabis in this research.

Behavioural evaluations

The rats were subjected to a battery of behavioural evaluations on the sixth day of the treatments, to assess locomotor activities, spatial memory and anxiety related behavior in the Open Field Test (OFT), the Y maze, and the Elevated Plus Maze (EPM) respectively.

Open field test (OFT): To assess the effects of the cannabis on exploratory activity, experimental animals were evaluated in the open-field paradigm. The paradigm is made of Perspex plastic with dimensions (40×60×50 cm) and the floor was divided into 25 equal squares by lines. Animals were individually placed in the centre of the apparatus and number rearings were counted and immobility period recorded in a 5 min session and all animals were monitored in a balanced design during the procedures (Imam et al, 2016[a&b]; Wahab et al, 2016).

Y maze

Y maze apparatus was used to assess the animals' spatial memory following oral cannabis. A stop watch was used to score the behaviours and all events were observed manually. A Y-maze is made up of three equally spaced arms, labelled as A, B, and C which are 120^{o} from each other, 41 cm long and 15 cm high. It was used to assess the spontaneous alternation in the rats. The floor of the apparatus is 5 cm wide and is levelled with saw shaves. Each rat was stationed in one of the arms and allowed to freely explore the apparatus. The sequence or consecutive entrance of the animals into the arms is termed an alternation.

The total number of arms entered minus two is termed spontaneous alternations, and the percentage alternation was calculated as [(actual alternations/maximum alternations) X100]. Five minutes was assigned as the test time limit for each of the animals in the Y-maze apparatus. Recorded data is the total arm entries indicate the total number of a single arm entered (e.g. ABCBCABACBC, contain 11 entries), from which the correct and wrong alternations are recorded (Imam et al, 2016[a]).

Elevated plus maze test (EPM)

For the assessment of anxiogenic activity of cannabis in rats, the elevated plus maze (EPM) paradigm was used. The EPM is made of two open arms (OA; 16 x 5 cm), two closed arms (CA; 16 x 5 12 cm) and was elevated (60 cm) above the floor. Rats were

individually placed at the centre of the EPM with heads facing the OA i.e., fear-inducing environment, allowed a 5 mins test session and the number of rearing was recorded.

Histopathology

The rats were anaesthetized 24 hours after the behavioural tests, decapitated and their brains excised and stored in 4% paraformaldehyde solution. The brains were subsequently embedded in paraffin, refrigerated, coronally sectioned into 8 μm sections of the frontal cortices (from Bregma 2 mm to 4 mm) and the hippocampal formation (from Bregma –2.5 mm to –4.5 mm) using a rotary microtome (MK 1110), and stained with Cresyl fast violet (CFV) for general neural architecture and nissl granulation following standard routine laboratory procedures (Bancroft & Gamble, 2008). An Olympus BX 51 microscope and a DP 12 digital camera were used to produce photomicrographs from all the sections.

Statistical analysis

Data recorded in this study were reported as mean ± standard error of mean. Y maze test's data was analyzed using one way analysis of variance (ANOVA) and Bonfferoni post-hoc analyses, while data from EPM and OFT were analyzed by T test. P value of ≤0.05 was considered statistically significant in all cases. The software package Graph Pad Prism was used for analysis and graphical representation of data.

RESULTS

Behavioural results

Activities and Anxiety-like behaviours

Locomotor activities and anxiety-like behaviours were assessed in the animals following cannabis exposure in OFT and EPM paradigms respectively. Cannabis markedly caused a significant ($p<0.05$) reduction in the rearing frequencies in both OFT and EPM (Figures 1 and 3) and an increase in the freezing period (sec.) in the OFT (Figure 2), when compared with the saline treated animals.

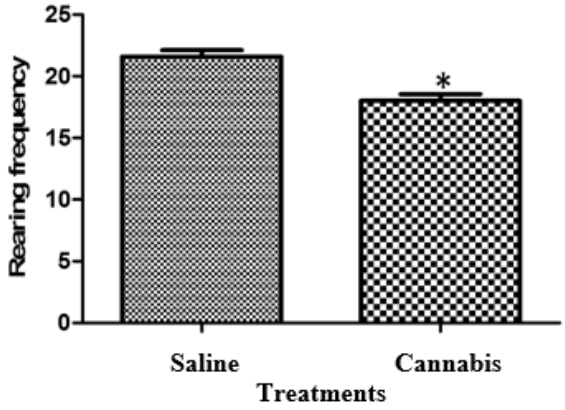

Figure 1: The effect of cannabis extract on the rearing frequency in the OFT paradigm, with a reduced rearing frequency recorded in the exposed animals. *p<0.05.

Spatial memory

The spatial memory was assessed using a standard Ymaze model. Cannabis significantly ($p<0.05$), caused a reduction in the percentage alternation, comparable to the scopolamine induced standard model in the treated animals (Figure 4).

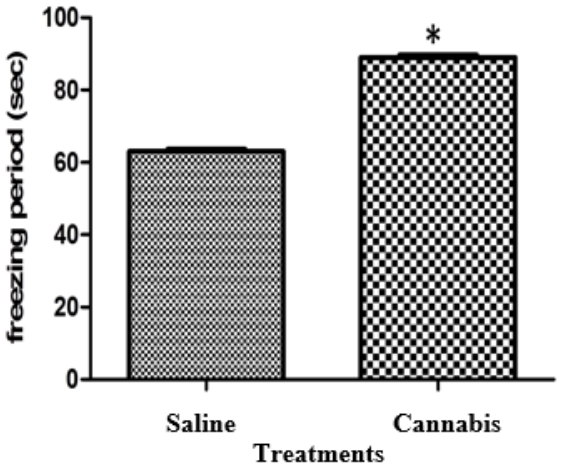

Figure 2: The effect of cannabis extract on the freezing period in the OFT paradigm, with increased freezing period (sec.) recorded in the exposed animals. *p<0.05.

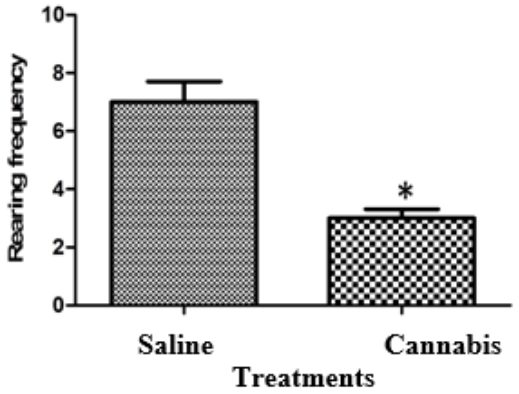

Figure 3: The effect of cannabis extract on the rearing frequency in the EPM paradigm, with a marked significant reduction in the rearing frequency in the exposed animals. *p<0.05.

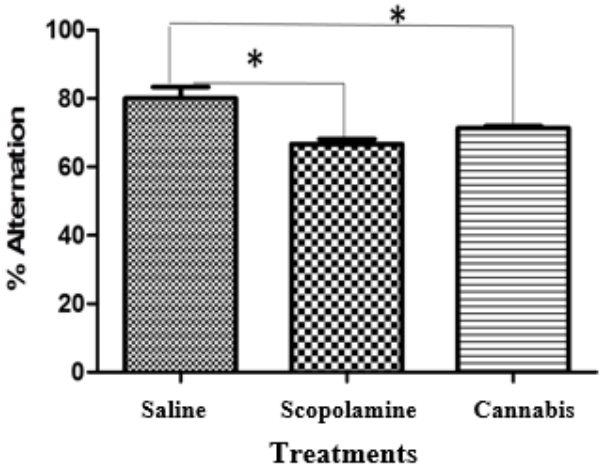

Figure 4: The effects of cannabis and scopolamine on the Percentage alternation in the Y maze paradigm, with a reduced percentage alternation in the exposed animals. *p<0.05.

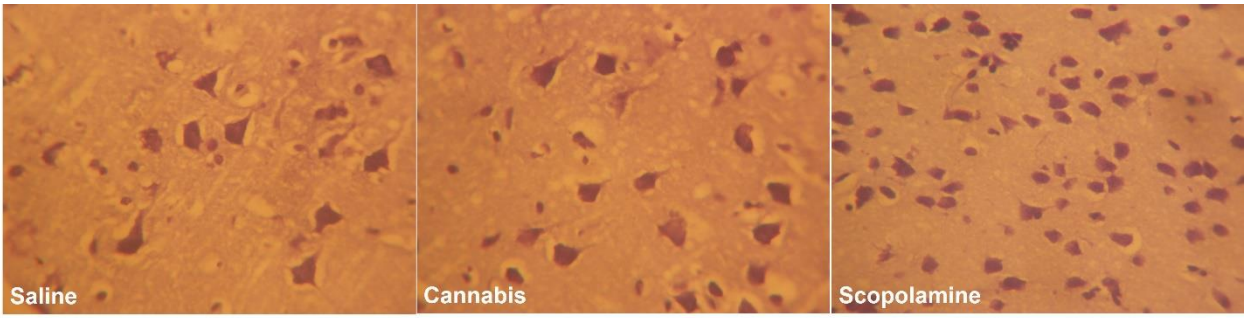

Figure 5: Representative photo micrograph of the sections of the frontal cortices of rats following saline, cannabis and scopolamine administrations. Showing: normal nissl distributions in the soma and unaffected proximal dendrites of the pyramidal neurons in the Saline; pyknotic and darkly stained soma of the pyramidal neurons in both Cannabis and Scopolamine exposed animals (CFV ×400).

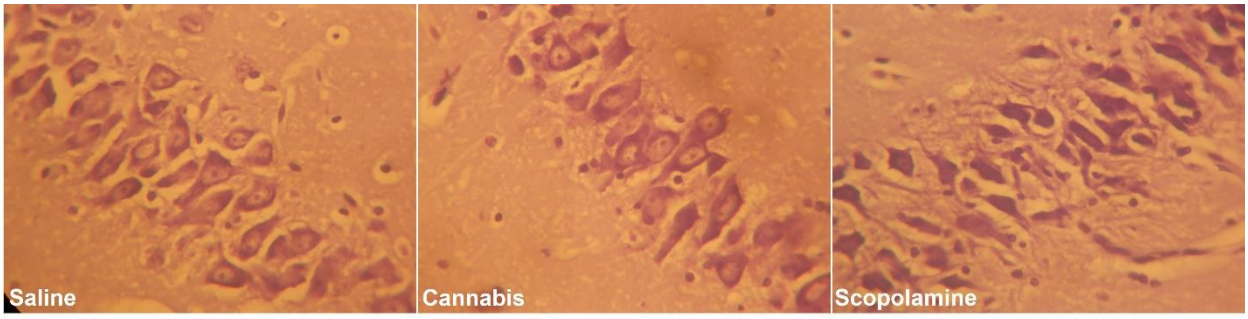

Figure 6: Representative photo micrograph of the sections of the hippocampal CA2 of rats following saline, cannabis and scopolamine administrations. Showing: normal nissl granulation in the integrative pyramidal neurons in Saline; contrasting stains, darkly stained soma and presence of dark neurons in Cannabis and Scopolamine exposed animals (CFV ×400).

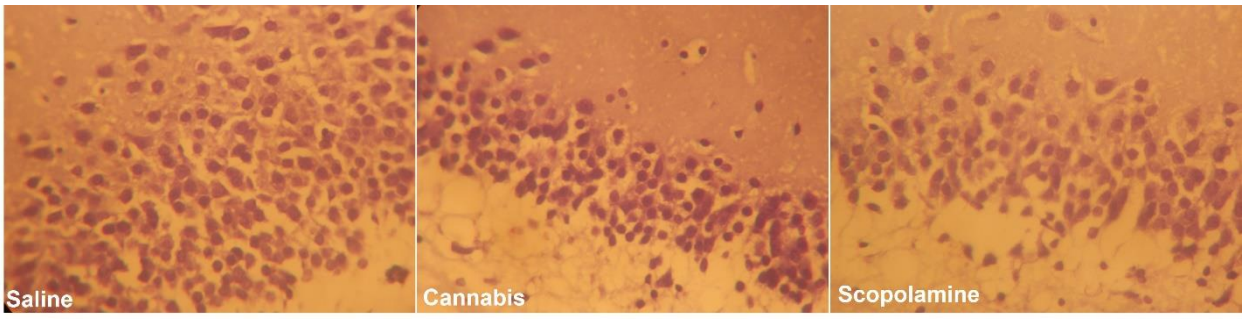

Figure 7: Representative photo micrograph of the sections of the dentate gyrus following saline, cannabis and scopolamine administrations. Showing: normal granulation and highly populated dentate granule cells in Saline; pyknotic soma and vacuolation in the surrounding neuropil in both Cannabis and Scopolamine exposed animals (CFV ×400).

Histopathological results

Sections of the Frontal cortices of the animals treated with cannabis and scopolamine, specifically the pyramidal cells at the deep pyramidal cell layer shows some degree of retraction of processes and shrunken perikaryon (Figure 5). Also, there are mark irregularities in the distributions of nissl granulation in the perikaryon and increased dark pyknotic nuclei, presence of dark neuron suggesting protein denaturation and neural degeneration (Figure 5).

Exposure to both cannabis and scopolamine also led to a marked change in the small pyramidal cells of the CA2, the outer layer was more affected, with darkened nuclei and clumped processes (Figure 6). In addition, some darkened, pyknotic nuclei are observed in the granular cells of the dentate gyrus with contrasting basophilia of some other granular cells nuclei, also observed are marked vacuolation. (Figure 7).

DISCUSSION

Indiscriminate use of cannabis and its products is a potential actor in the cumulative causal factors for some neuropsychiatric disorders, like schizophrenia and contribute to worsening outcomes in user with prior psychosis incidences (David, 2013), but its abuse still increase across boundaries. The growing perceptions of no harm and medicinal potentials (Tzadok et al, 2016; Lynch, 2016; Velasco et al, 2016; Juknat et al, 2016) in cannabis use has boosted its abuse over time.

In this study, cannabis was recorded to impair exploratory activities in the treated animals, as seen in the reduced rearing frequencies in both OFT and EPM

paradigms. This is suggestive of the impairment of locomotory and seeking activities by acute cannabis usage which is in line with previous studies, where a reduced line crossing and explorative activities were reported (Keeley et al, 2015). Such effects may be linked to a possible inhibition of transmission of neural signals through the basal ganglia and cerebellum.

The reduced exploratory activities observed with cannabis, is also strengthened with the high freezing period, or the immobility period recorded in the OFT. This can point to the possible anxiogenic property of cannabis, in support of earlier studies where a reduced locomotor activities and increased anxiety like behaviours have been reported following cannabis exposure (Okon et al, 2014; Keeley et al, 2015).

These impaired activities in exploratory behaviours and the displayed anxiety related behaviours with cannabis treatment can be supported by its induced severity in psychotic symptoms, high dependency/abuse status and impaired striatal dopamine reported in users (Bloomfield et al, 2016; Ramaekers et al, 2016), implicating impaired psycho-cognitive and psycho-motor behaviours. It has also been demonstrated that acute exposure to the primary psychoactive component THC, causes transient, acute psychotic reactions, the extent of which are related to the degree of cognitive impairment or anxiety.

It is noteworthy that, both animal and human studies indicated that adolescent cannabis exposure is more vulnerable to impaired neurocognitive function than older individuals, and such impairment can persist after abstinence (Fontes et al, 2011). However, only a small percentage of this vulnerable group thought that cannabis smoking puts users at a greater risk (Miech et al, 2014).

Cannabis like scopolamine, also reduced percentage alternation of the exposed animals in the Y maze, which is an indication of impaired spatial memory or spatial capacity. We have previously reported that cannabis impaired working memory, short term and long term memory, by delaying escape latency in the Morris water maze (MWM) in rats (Imam et al, 2016), and such was supported by reports from other researchers (Omar et al, 2013; Renard et al, 2014; Keeley et al. 2015).

Neurocognitive impairments associated with cannabis use have received great attention in the recent past and outcomes include, impaired ability to relearn (Desirée et al, 2016), impaired prospective memory, verbal immediate and delayed recall as well as visual recognition in individuals without prior neuropsychiatric disorders (Schoeler et al, 2016; Christian et al, 2016), implicating a wider coverage of impaired neurocognitive performances (Valerie et al, 2016). These neurocognitive effects persist after withdrawal, but little is known of these effects (Bahreh et al, 2016).

Strengthening the impaired psycho-cognitive behaviours above, is the partial deleterious effects observed in the frontal cortices, hippocampal CA2 and the dentate gyrus neural architectures following cannabis exposure in this study. Such reports are similar to degenerative effects in various brain regions and marked neuronal damage in rats earlier reported (Omar et al, 2014; Odokuma and OgborOmorie, 2016). Other studies have associated the long term heavy use of cannabis with gross anatomical abnormalities in the hippocampus and the amygdala (Solowij et al, 2013; Battistella et al, 2014) and other brain regions (Hester et al, 2009; Smith et al, 2010), that are reported to be rich in cannabinoid receptors.

Other than the effects of cannabis on neuronal morphology, there are also some evidences that cannabis use is implicated in impaired neural connectivity. For example, decreased connectivity in the right hippocampus finbrae (fornix) and the splenium of the corpus callosum and the commissural fibers was reported from the MRI of heavy long term cannabis users (Orr et al., 2013) and altered neural activity both in the resting state and during cognitive testing (Batalla et al, 2014). These further suggest that there is a strong structural background to the various neurocognitive impairments seen in cannabis users.

The frontal cortices and the hippocampus which play major roles in memory and other executive function appear to very much affected than other parts in this study. Cannabis was reported to reduce basal dendrite arborization in pyramidal neurons in the PFC of adult rats (Justine et al, 2016), induce gray-matter alteration in prefrontal cortices (Batalla et al, 2013) and causes abnormal connectivity, in the hippocampal afferent fibers (Zalesky et al, 2012). Considering the well-established role of both the hippocampus and the FC in learning and memory (Churchwell and Kesner, 2011), the structural perturbation of these two structures may be the basis for the emergence of neurocognitive deficits observed after acute administration of cannabis in this study.

Overall, our findings demonstrated that repeated or acute cannabis use could impair the neuronal morphology and architecture of pyramidal cells of CA2 area and granule cells of dentate gyrus, and consequently hippocampal-dependent cognitive and emotional behaviors.

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