

EFFECT OF AQUEOUS EXTRACT OF *CANNABISSATIVA* LEAF ON THE MOTOR COORDINATION USING THE HANGING WIRE AND OPEN FIELD NEUROBEHAVIOURAL TESTS IN MALE WISTAR RATS

ABSTRACT

Cannabis sativa is a commonly abused drug especially among younger people in society. The cerebellum is located at the back of the brain, immediately inferior to the occipital and temporal lobes within the posterior cranial fossa. The study was designed to show the effect of aqueous leave extract of cannabis sativa on the performance of male Wistar rats in the hanging wire and open field neurobehavioural tests. A total of 40 Wistar rats were used and grouped into five groups. Group I received distilled water for 28 days. Group II, III, IV and V served as the low, high, low dose recovery and high dose recovery group respectively. Group II were administered with 10mg/kg body weight of cannabis sativa leave aqueous extract for 28 days. Group III were administered with 20mg/kg body weight of cannabis sativa leave aqueous extract for 28 days. Group IV was administered with 10mg/kg body weight of cannabis sativa leave aqueous extract for 28 days and were allowed for further 28 days without any administration while group V received 20mg/kg body weight of cannabis sativa for 28 days and were allowed for further 28 days without administration. group IV and V represent the recovery group. Group I, II and III were sacrificed a day after their last intubation. The result of the study showed that

Keywords: Cannabis sativa; hanging wire test; open field test; motor coordination

Introduction

Cannabis sativa is a commonly abused plant due to its high content of the psychoactive compound (Lucas *et al.*, 2018). Though cannabis has been used for medical purposes due to

its antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective properties, its adverse consequences should not be underestimated (Ford *et al.*, 2017 ; da Silva *et al.*, 2018).

Cannabis sativa is an annual herbaceous flowering plant indigenous to Eastern Asia, but now of cosmopolitan distribution due to widespread cultivation. It has been cultivated throughout recorded history, used as a source of industrial fiber, seed oil, food, recreation, religious and spiritual moods and medicine. Each part of the plant is harvested differently, depending on the purpose of its use. The flowers of *Cannabis sativa* are short-day flowering plants, with staminate (male) plants usually taller and less robust than pistillate (female) plants (United Cannabis Seeds 2021). The flowers of the female plant are arranged in racemes and can produce hundreds of seeds. Male plants shed their pollen and die several weeks prior to seed ripening on the female plants. Under typical conditions with a light period of 12 to 14 hours, both sexes are produced in equal numbers because of heritable X and Y chromosomes (Clark and Merlin, 2013). Although genetic factors dispose a plant to become male or female, environmental factors including the diurnal light cycle can alter sexual expression (Schaffner, 2020).

Understanding potential toxicity is crucial for safety considerations, especially if the plant extract is used in traditional medicine or as a dietary supplement. Cannabis use is common among adolescents and young adults, but the long-term consequences of such use are a topic of debate. Cannabis use typically starts during early adolescence and peaks when users are in their mid-20s (Hasin *et al.* 2015). In a large US survey, 7.4% of adolescents reported cannabis use during the past month and 13.1% during the past year (Azofeifa 2016). Cannabis use can have adverse health effects, including increased risks for lung, cardiovascular, and periodontal diseases (Gordon *et al.* 2013; Jouanjus *et al.* 2017). Its effects on development of cognitive and affective dysfunction, however, have been less conclusive. An initial study reported that cannabis use, particularly during adolescence, contributes to a lasting neurocognitive decline including an 8-point drop in IQ from childhood to adulthood (Jackson *et al.* 2016). More recent studies, however, do not support this conclusion. For example, cannabis users perform worse on cognitive tests than non-users, but the performance of users is comparable to their non-using twins (Meier *et al.* 2018).

Receptors for THC and other cannabinoid compounds are present in the brain, especially in the frontal cortex, basal ganglia, cerebellum, and limbic regions. Cannabinoid action in the basal ganglia and cerebellum probably account for the effect on psychomotor control (John, 2003). Sensorimotor signals can be used to monitor and refine ongoing movements, while generalized changes in behavioral state, including arousal and levels of locomotor activity influence sensory processing and perception (McGinley et al., 2015; Schneider and Mooney, 2015; Vinck et al., 2015;Pakan et al., 2016). Both locomotor activity and arousal modulate delay eyeblink conditioning, a form of cerebellum-dependent associative learning (Albergaria et al., 2018).

Cannabinoids are profound modulators of behavioral state, across species (Mackie, 2007; Oakes et al., 2017; Luchtenburg et al., 2019). Acutely, cannabis and THC produce a range of effects on several neurocognitive and pharmacological systems. These include effects on executive, emotional, reward and memory processing via direct interactions with the endocannabinoid system and indirect effects on the glutamatergic, GABAergic and dopaminergic systems (Bloomfield, 2019).Blázquez *et al.*, (2020), found that D 9 - tetrahydrocannabinol, the psychoactive ingredient of cannabis, disrupts autophagy selectively in the striatum, a brain area that controls motor behavior, both in vitro and in vivo. Boosting autophagy, either pharmacologically (with temsirolimus) or by dietary intervention (with trehalose), rescued the D 9 -tetrahydrocannabinol-induced impairment of motor coordination in mice. Taken together, these findings identify inhibition of autophagy as an unprecedented mechanistic link between cannabinoids and motor performance and suggest that activators of autophagy might be considered as potential therapeutic tools to treat specific cannabinoid-evoked behavioural alterations.

Of concern are the effects of cannabis use on decision-making, especially when it involves risk-taking. Self-report questionnaires and laboratory risk-taking tasks have demonstrated differences between cannabis users and non-users (Burggren *et al* (2019). Adolescence and teens who engage in heavy marijuana use often show disadvantages in neurocognitive performance, macrostructural and microstructural brain development, and alterations in brain functioning. It remains unclear whether such disadvantages reflect pre-existing differences that lead to increased substances use and further changes in brain architecture and behavioral

outcomes (Jacobus and Tapert, 2014). Adult studies of marijuana use often find subtle decreases in performance compared to controls in cognitive domains such as attention, memory, and processing speed; such effects have been discussed as transient in the literature given limited group differences after prolonged abstinence from marijuana (Grant *et al.*, 2003; Pope *et al.*, 2001). Ongoing cognitive development in the domains of memory and executive functioning, and particularly in specialized functions like cognitive control, is not only tightly associated with adolescence and neocortical tissue maturation, but is likely to have implications for school performance and engagement in risk/reward behaviors (Casey *et al.*, 2008).

One of the earliest studies on the effects of marijuana on adolescent neurocognitive development evaluated verbal and nonverbal memory performance in cannabis-dependent adolescents (ages 14 to 16) compared to matched controls (Schwartz *et al.*, 1989). Schwartz and colleagues found that short-term memory impairment persisted after six weeks of monitored abstinence. In contrast, Teichner and colleagues (2000) found no relationship between marijuana use severity and cognitive performance among cognitively impaired and unimpaired adolescents referred for drug treatment.

Takagi and colleagues found that cannabis users (ages 13–24) performed worse on measures of immediate and delayed verbal memory compared to community controls. In a similar study by this team of investigators, no differences between cannabis users and community controls were found on measures of executive functioning (Takagi *et al.* 2011). Similarly, Gonzalez and colleagues (2012) found differences on immediate and delayed recall among young adult cannabis users (approximately age 20) compared to non-using controls, however no differences were observed on measures of impulsivity. Despite no group differences on impulsivity, the authors found that worse performance on a decision-making task was related to more cannabis use disorder symptoms. Solowij and colleagues looked at 181 adolescents (ages 16–20) and found that cannabis users performed worse on learning and recall, and poorer performance was related to severity, frequency, and age of initiation of cannabis use. Chronic cannabis use has also been associated with reduced gray matter volumes and memory deficits in cohorts comprising both PWH and seronegative controls (Cristiani *et al.*, 2004; Chang *et al.*, 2006; Battistella *et al.*, 2014; Thames *et al.*, 2017). Recent data from a group

suggest that a lifetime history of cannabis use disorders lowers the odds of neurocognitive impairment in PWH (Watson *et al.*, 2020) and may even promote “youthful” and resilient neurocognitive abilities among adults aging with HIV (Saloner *et al.*, 2019b).

Increased ambulation and exploratory activities as well as decreased immobilisation on paradigms such as open field maze, hole board maze and white and black box indicated intact motor system and low anxiety. While decrease in activities suggest an expression of anxiogenic state (Rice *et al.*, 1981). Rodents display rearing behaviour by standing on their hind limbs with the forelimbs raised into the air or on the wall of the maze. This behaviour allows the animals to evaluate the potential danger of the immediate space and how to find an escape-route. Increase in this behaviour indicated fear, agitation and anxiety to leave the maze. Cannabis-diet decreased rearing behaviour in all groups fed except for group fed low quantity cannabis-diet (1 % cannabis-diet). This may suggest that increased cannabis consumption may reduce anxiety, even though the association between rearing behaviour and anxiety remains controvertible (Seibenhener and Wooten, 2015; Animal Research International (2019). Cannabis-diet fed groups displayed slightly higher locomotor/exploratory activity which is consistent with low levels of anxiety. Our observation was in contrast with the findings of Okon *et al.* (2014) which reported a dose-dependent decrease in locomotion and exploratory behaviour. Findings in the light/dark transition box showed that cannabis-diet fed mice demonstrated a striking and strong affinity for well illuminated open spaces suggesting that the *C. sativa* has anxiolytic effect. Rodents naturally associate more with dark spaces as it offers hiding spots and safety. Increased attraction to dark space than light space indicates anxiogenic condition. In this study, the cannabis-treated mice had exploratory activity not significantly different from the control, which is in contrast with a previous report (Harte-Hargrove and Dow-Edwards 2012).

The neurobehavioral assessments conducted following the administration of *Cannabis sativa* showed that there was no significant difference in freezing and center square entries, while there was a significant difference in the number of lines crossed when groups B and D were compared to the control. Rearing frequency in group C was also relatively different from the control. The changes observed in the decline in the number of lines cross suggests the role

of cannabis in influencing locomotor activity and also the increase in rearing frequency directly points to the role of cannabis in initiating anxiety-like behaviors. Y-Maze test was used to test for cognition, spatial learning, and memory assessment in rats exposed to *Cannabis sativa*. There was no significant difference in the total arm entries and spontaneous alteration across all groups, but there was an observable increase in group C when compared with control (Yinka *et al.*, 2023).

Following treatment with Cannabis for 21 days after which behavioral indices were assessed on the 22nd day revealed a significant reduction in the number of lines crossed in groups B (10 mg/kg of *Cannabis sativa*) and D (100 mg/kg of *Cannabis sativa*) when compared to the control and significantly increased rearing frequency in group C (50 mg/kg of *Cannabis sativa*) when compared with the control, while there was no significant difference in freezing and center square entries (Yinka *et al.*, 2023). There is a reduction in locomotor activity (line crossing), strong forelimb support (hanging wire) and slower vestibular response (negative geotaxis value) in the cannabis exposed rats (group B) compared to the control group A, group C values showed a recovery of these deficits, except for the fore limb support (27.81 ± 19.13) where they showed greater strength (Osinubi *et al.*, 2019).

In the large open field test, there were no main effects or interactions involving smoke exposure condition on total distance traveled, distance traveled in the border zone, distance traveled in the center zone, number of center zone entries, or time spent in the center zone (Bruijnzeel *et al.*, 2019). There were no differences in the total number of horizontal or vertical beam breaks between the air-control rats and the cannabis rats before the onset of the smoke exposure sessions. In both groups, there was an *effect of time* on horizontal and vertical beam breaks (Bruijnzeel *et al.*, 2016). The cannabis rats and air-control rats received vehicle or rimonabant and somatic withdrawal signs were recorded.

Bruijnzeel *et al.*, (2019) reported an increase in weight across all groups of rats, males weighed more than the females. They noted a significant increase in body weight across smoke exposure days and the magnitude of this increase was significantly greater in males than females. The rats were exposed to cannabis smoke or air for 8 weeks and during this period both groups gained the same amount of weight (Bruijnzeel *et al.*, 2016).

The cerebellum is a crucial part of the brain responsible for motor coordination, balance, and cognitive functions. Researching the effects of cannabis leaf extract on the cerebellum contributes to our understanding of neurobiology and can provide insights into how various substances affect brain function. If the plant extract is being considered for use in pharmaceuticals or herbal remedies, research on its effects on the cerebellum is essential for regulatory approval and ensuring consumer safety. Studying the effects of leaf extract on the cerebellum of rats is significant because it contributes to our understanding of neuroscience, may reveal potential therapeutic benefits or risks associated with the extract, and can inform regulatory decisions regarding its use in medicine or other applications.

Materials Used in the Study

Materials used includes Adult Wistar rats, Cannabis Sativa leaves, distilled water, well-ventilated cages, weighing balance, syringes, dissecting kit, specimen containers, cotton wool, methylated spirit, saw dust which will serve as the animal bedding will be used for the study.

Ethical Consideration

Ethical clearance was sought and obtained from the Research Ethics Committee of the Faculty of Basic Health Sciences, Nnamdi Azikiwe University Awka, Anambra State Nigeria

Sourcing and Handling of Cannabis Sativa

Fresh leaves of Cannabis sativa was obtained from the locals and authenticated at botany department, Nnamdi Azikiwe University, Awka.

Sourcing and Handling of Wistar Rats

The rats were obtained from the animal house of Physiology department, Nnamdi Azikiwe University, Nnewi campus. The animals were housed within the standard facilities of a well-ventilated animal house and maintained on a standard of rodent pallets and water ad libitum under standard laboratory conditions of lighting and moderate temperature.

Lethal Dose (LD50) of Cannabis Sativa Determination

Lethal Dose (LD50) of *Cannabis Sativa* was carried out according to Lorke's method.

Experimental Design

A total of 40 adult Wistar Rats weighing between 180g-200g was used for this study. Fifteen (15 rats) was used for **LD50** determination and 25 experimental rats for the study proper with 5 rats per group.

Group I: received distilled water for 28 days; **Group II:** received low dose for 28 days; **Group III:** received high dose for 28 days; **Group IV:** received low dose for 28 days and allowed a recovery period of 28 days; **Group V:** received high dose for 28 days and allowed a recovery period of 28 days

Animal Sacrifice and Tissue Collection Technique

At the end of the administration period, the rats were anesthetized and sacrificed by cervical dislocation. The brain tissues were carefully removed from the skull and trimmed of the surrounding tissues, and immediately fixed in 10% bouins fluid for 24 hours. After which the Cerebellum was excised separately for histological processing using H&E. The sections were studied for histopathological changes and micrographs of histological sections were taken for comparative studies between the various experimental groups and the control group. The remains from the sacrificed rats were buried.

3.10 Statistical Analysis

The data were presented as Mean \pm SEM of 5 rats in each group, subjected to one-way Anova test using Turkey's post-test to show differences between the mean values of all groups. A value of $p < 0.05$ will be interpreted as statistically significant.

RESULTS AND DISCUSSION OF FINDINGS

Result of open field test

The result of open field test presented in table 1 below evaluated 6 different parameters. Summarily, administration of CS did not significantly change the out-come of the open field test at the final stage compared to the initial. On the average rearing was more frequent in the

experimental groups compared to the control, although the difference was not statistically significant.

The same goes for grooming, rats had a statistically non-significant grooming tendencies at FINAL following CS administration compared to the initial.

Line arising was non-significantly less frequent at the final state compared to the initial stage. This implies the animals moved less within the chamber. This is corroborated by the higher duration of freezing recorded at the final stage compared to the initial stage.

Urination and defecation were relatively similar for different groups of rats at the final stage compared to the initial, except in the control group A (Faeces) and experimental groups B & C (Urination) with significantly higher frequency of defecation and urination respectively.

Table 1. List of parameters used for Urination and defecation

| PARAMETER | GROUP | INITIAL | FINAL | PVALUE |
|---------------|-------|--------------|--------------|--------|
| Rearing | A | 7.2 ± 1.92 | 9.8 ± 3.49 | 0.281 |
| | B | 7.2 ± 1.83 | 9.0 ± 2.23 | 0.286 |
| | C | 6.6 ± 1.14 | 10.4 ± 4.15 | 0.149 |
| | D | 16.6 ± 7.40 | 7.0 ± 2.23 | 0.033 |
| | E | 7.2 ± 5.80 | 7.2 ± 3.83 | 1.000 |
| Grooming | A | 1.0 ± 0.71 | 1.2 ± 0.45 | 0.704 |
| | B | 0.6 ± 0.55 | 1.0 ± 1.22 | 0.477 |
| | C | 0.8 ± 0.45 | 1.0 ± 0.71 | 0.374 |
| | D | 0.2 ± 0.45 | 1.6 ± 1.14 | 0.108 |
| | E | 0.4 ± 0.55 | 1.0 ± 0.22 | 0.208 |
| Line crossing | A | 39.2 ± 8.53 | 24.4 ± 7.63 | 0.077 |
| | B | 27.2 ± 8.58 | 25.0 ± 11.53 | 0.727 |
| | C | 26.8 ± 6.14 | 30.4 ± 3.36 | 0.278 |
| | D | 33.4 ± 10.09 | 29.8 ± 7.56 | 0.530 |
| | E | 32.8 ± 9.47 | 32.6 ± 8.38 | 0.976 |
| Faeces | A | 1.2 ± 1.30 | 4.8 ± 1.92 | 0.041 |
| | B | 2.80 ± 2.28 | 1.80 ± 1.30 | 0.546 |
| | C | 1.80 ± 1.64 | 2.40 ± 1.95 | 0.634 |
| | D | 4.2 ± 1.64 | 2.2 ± 0.49 | 0.389 |
| | E | 2.2 ± 2.05 | 0.4 ± 0.89 | 0.088 |
| Urine | A | 1.8 ± 1.79 | 1.8 ± 0.84 | 1.000 |
| | B | 4.4 ± 2.07 | 2.0 ± 1.00 | 0.024 |
| | C | 4.8 ± 1.09 | 1.4 ± 1.14 | 0.000 |
| | D | 2.6 ± 2.61 | 4.2 ± 5.21 | 0.599 |

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|----------|---|--------------|--------------|-------|
| | E | 1.0 ± 0.41 | 3.0 ± 1.87 | 0.089 |
| Freezing | A | 41.2 ± 12.83 | 50.8 ± 12.99 | 0.311 |
| | B | 37.2 ± 17.14 | 61.2 ± 39.36 | 0.291 |
| | C | 29.6 ± 9.81 | 20.4 ± 2.60 | 0.126 |
| | D | 37.8 ± 17.37 | 39.8 ± 9.68 | 0.867 |
| | E | 35.6 ± 16.09 | 54.8 ± 11.37 | 0.052 |

Amaza et al (2013) observed physical changes which include hyperactivity, increase in appetite as well as increase in weight, this is due to the fact that endocannabinoids in the hypothalamus activate cannabinoid receptors that are responsible for maintaining food intake and also cannabis Sativa has acute appetite enhancing effects, thereby increasing body weight in experiment model in group III except in group II Wistar rats. The result for open field test in this study shows cannabis Sativa did not significantly change the outcome of the open field test at the final stage compared to the initial. This is similar to a study conducted on prospective memory evaluation for undergraduate between the ages of 18-24-year old, where no difference in self-reported prospective memory was identified, cannabis users did recall fewer location action combination during the video (Meier et al, 2012).

Animal moved less within the chamber of line crossing. This is corroborated by the higher duration of freezing recorded at the final stage compared to the initial stage. This report is in terms with Rice and Colleague who observed decreased immobilization on paradigms such as open field maze, hoic board maze and white and black box indicated intact motor system and low anxiety. The decrease in activities suggest an expression of anxiogenic state (Rice et al 1981). Ingestion of whole cannabis plant may not adversely influence neurobehavioral patterns in mice. There were no correlation between the mild change in behavioral pattern and oxidative stress differentials in mice that consumed cannabis within the study period, this report is very consistent generally with the report of this study that the administration of CS did not significantly change the outcome of the open field test and hanging wire at the final stage compared to the initial stage and similarly oxidative stress report (Akinola et al 2019).

Yinka and friends in 2023 accessed behavioral indices of cannabis administration for 21 days their results revealed a significant reduction in the number of line crossed in 10mg/kg which is in conformity with our study which we reported line crossing to be non-significantly less at the final

stage when compared to the final. (Yinka et al 2023) Osinubi et.,al 2019 also reported reduction in locomotive activity which agrees to this study. In the same study which coincides with this study, Yinka reported an increased rearing frequency in model group, no difference in freezing when compared to control. This study reported more rearing as more frequent in model group when compared to control, and animal move less within the chamber which corroborate high duration of freezing at the final stage (Yinka et.,al 2023).

RESULT OF HANGING WIRE NEUROBEHAVIOURAL TEST

TABLE 2: RESULT OF HANGING WIRE TEST

| GROUP | INITIAL | FINAL | P VALUE |
|-------|----------------|---------------|---------|
| A | 34.80 ± 2.95 | 38.75 ± 37.51 | 0.267 |
| B | 89.00 ± 34.95 | 45.60 ± 55.09 | 0.088 |
| C | 72.6 ± 66.13 | 90.2 ± 59.73 | 0.335 |
| D | 60.00 ± 65.96 | 39.20 ± 28.54 | 0.195 |
| E | 102.40 ± 30.22 | 19.60 ± 15.85 | 0.000 |

Results are presented as Mean ± SD of 5 rate in each group. P < 0.05 is considered statistically significant.

In this study, the result of hanging wire test shows that rats spent significantly different time in the final stage of the experiment compared to the initial stage of the experiment, this report is in line with Osinubi and colleagues (2019) work where rats have higher hanging wire value compared to the initial.

Conclusion

The administration of cannabis Sativa did not significantly change the outcome of the open field test at the final stage compared to the initial but group E showed significant decrease in hanging time following exposure to *cannabis sativa*.

REFERENCES

Abdel-Salam OME, Kha 0. Abdel-Salam OME, Khadrawy YA, Youness ER, Mohammed NA, AbdelRahman RF, Hussein JS (2014). Effect of a single intrastriatal rotenone injection on oxidative stress and neurodegeneration in the rat brain. *Comparative Clinical Pathology*; 23: 1457-1467. 41.

Albergaria C, Silva NT, Pritchett DL, Carey MR. 2018. Locomotor activity modulates associative learning in mouse cerebellum. *Nature Neuroscience* 21:725–735. DOI: <https://doi.org/10.1038/s41593-018-0129-x>, PMID: 29662214

Abdel-Salam OME, Youness ER, Mohammed NA, Abd El-Moneim OM, Shaffie N (2019). Citicholine protects against tramadol-induced oxidative stress and organ damage. *Reactive Oxygen Species*; 7(20): 106- 120. 40.

Azofeifa A (2016) National estimates of marijuana use and related indicators—National Survey on Drug Use and Health, United States, 2002–2014. *MMWR Surveillance Summaries* 65. [PubMed] [Google Scholar]

Battistella G, Fornari E, Annoni JM, Chtioui H, Dao K, Fabritius M, Favrat B, Mall JF, Maeder P, Giroud C (2014). Long-Term Effects of Cannabis on Brain Structure. *Neuropsychopharmacology*; 39:2041–2048.

Blázquez C, Ruiz-Calvo A, Bajo-Grañeras R, Baufreton JM, Resel E, Varilh M, Pagano Zottola AC, Mariani Y, Cannich A, Rodríguez-Navarro JA, Marsicano G, Galve-Roperh I, Bellocchio L, Guzmán M (2020). Inhibition of striatonigral autophagy as a link between cannabinoid intoxication and impairment of motor coordination. *Elife*; 10;9:e56811.

Bloomer RJ, Butawan M, Smith NJG (2018). Chronic marijuana smoking does not negatively impact select blood oxidative stress biomarkers in young, physically active men and women. *Health*; 10(07): 960- 970. 34.

Bloomfield MAP, Hindocha C, Green SF, Wall MB, Lees R, Petrilli K, Costello H, Ogunbiyi MO, Bossong MG, Freeman TP (2019). The neuropsychopharmacology of cannabis: A review of human imaging studies. *Pharmacology and Therapeutics*; 195:132-161.

Burggren AC, Shirazi A, Ginder N, London ED (2019). Cannabis effects on brain structure, function, and cognition: considerations for medical uses of cannabis and its derivatives. *American Journal of Drug and Alcohol Abuse*; 45(6):563-579.

Bruijnzeel AW, Knight P, Panunzio S, Xue S, Bruner MM, Wall SC, Pompilus M, Febo M, Setlow B. Effects in rats of adolescent exposure to cannabis smoke or THC on emotional behavior and cognitive function in adulthood. *Psychopharmacology (Berl)*. 2019 Sep;236(9):2773-2784. doi: 10.1007/s00213-019-05255-7. Epub 2019 May 2. PMID: 31044291; PMCID: PMC6752736.

Bruijnzeel AW, Qi X, Guzhva LV, Wall S, Deng JV, et al. (2016) Behavioral Characterization of the Effects of Cannabis Smoke and Anandamide in Rats. *PLOS ONE* 11(4): e0153327. <https://doi.org/10.1371/journal.pone.0153327>

Chang L, Cloak C, Yakupov R, Ernst T (2006). Combined and Independent Effects of Chronic Marijuana Use and HIV on Brain Metabolites. *Journal of Neuroimmune Pharmacology*; 1:65–76

Cristiani SA, Pukay-Martin ND, Bornstein RA (2004). Marijuana use and cognitive function in HIV-infected people. *The Journal of Neuropsychiatry and Clinical Neurosciences*; 16:330–335

Dykstra, M. J., and Reuss, L. E. (2003). *Biological Electron Microscopy: Theory, Techniques, and Troubleshooting*, 2nd Edn. Boston, MA: Springer US.

Eraso-Pichot A, Pouvreau S, Olivera-Pinto A, Gomez-Sotres P, Skupio U, Marsicano G (2023). Endocannabinoid signaling in astrocytes. *Glia*; 71(1):44-59.

Ford TC, Hayley AC, Downey LA, and Parrott AC (2017). Cannabis: an overview of its adverse acute and chronic effects and its implications. *Current Drug Abuse Reviews*; 10(1): 6–18.

Fu Z, Zhao P-Y, Yang X-P, Li H, Hu S-D, Xu Y-X and Du X-H (2023), Cannabidiol regulates apoptosis and autophagy in inflammation and cancer: A review. *Frontiers in Pharmacology*; 14:1094020.

Harte-Hargrove, L. C. and Dow-Edwards, D. L. (2012). Withdrawal from THC during adolescence: sex differences in locomotor activity and anxiety. *Behavioural Brain Research*; 231(1): 48 – 59.

Hasin DS, Wall M, Keyes KM, Cerdá M, Schulenberg J, O'Malley PM, Galea S, Pacula R, Feng T (2015) Medical marijuana laws and adolescent marijuana use in the USA from 1991 to 2014: results from annual, repeated cross-sectional surveys. *The Lancet Psychiatry* 2: 601–608. [PMC free article] [PubMed] [Google Scholar]

Ignatowska-Jankowska B, Jankowski MM, Swiergiel AH. Cannabidiol decreases body weight gain in rats: involvement of CB2 receptors. *Neurosci Lett*. 2011 Feb 18;490(1):82-4. doi: 10.1016/j.neulet.2010.12.031. Epub 2010 Dec 21. PMID: 21172406.

Jacobus J, Tapert SF (2014). Effects of cannabis on the adolescent brain. *Current Pharmaceutical Design*; 20(13):2186-93.

Jackson NJ, Isen JD, Khoddam R, Irons D, Tuvblad C, Iacono WG, McGue M, Raine A, Baker LA (2016) Impact of adolescent marijuana use on intelligence: Results from two longitudinal twin studies. *Proceedings of the National Academy of Sciences* 113: E500–E508. [PMC free article] [PubMed] [Google Scholar]

Jouanjus E, Raymond V, Lapeyre-Mestre M, Wolff V (2017) What is the current knowledge about the cardiovascular risk for users of cannabis-based products? A systematic review. *Current atherosclerosis reports* 19: 26. [PubMed] [Google Scholar]

Kelly R, Joers V, Tansey MG, McKernan DP, Dowd E (2020). Microglial Phenotypes and Their Relationship to the Cannabinoid System: Therapeutic Implications for Parkinson's Disease. *Molecules*; 21;25(3):453.

Longoria V, Parcel H, Toma B, Minhas A, Zeine R (2022). Neurological Benefits, Clinical Challenges, and Neuropathologic Promise of Medical Marijuana: A Systematic Review of Cannabinoid Effects in Multiple Sclerosis and Experimental Models of Demyelination. *Biomedicines*; 10(3):539.

Lucas CJ, Galettis P, and Schneider J (2018). The pharmacokinetics and the pharmacodynamics of cannabinoids. *British Journal of Clinical Pharmacology*; 84(11): 2477–2482.

Luchtenburg FJ, Schaaf MJM, Richardson MK. 2019. Functional characterization of the cannabinoid receptors 1 and 2 in zebrafish larvae using behavioral analysis. *Psychopharmacology* 236:2049–2058. DOI: <https://doi.org/10.1007/s00213-019-05193-4>, PMID: 30820632

McGilveray IJ (2005). Pharmacokinetics of cannabinoids. *Pain Research and Management*; 10 Suppl A:15A-22A. 31.

Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RS, McDonald K, Ward A, Poulton R, Moffitt TE (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of National Academy of Science, USA*.

Meier MH, Caspi A, Danese A, Fisher HL, Houts R, Arseneault L, Moffitt TE (2018) Associations between adolescent cannabis use and neuropsychological decline: a longitudinal co-twin control study. *Addiction* 113: 257–265. [PMC free article] [PubMed] [Google Scholar]

Oakes MD, Law WJ, Clark T, Bamber BA, Komuniecki R. 2017. Cannabinoids activate monoaminergic signalling to modulate key *C. elegans* Behaviors. *The Journal of Neuroscience* 37:2859–2869. DOI: <https://doi.org/10.1523/JNEUROSCI.3151-16.2017>, PMID: 28188220

Okon, V. E., Obembe, A. O., Nna, V. U. and Osim, E. E. (2014). Long-term administration of Cannabis sativa on locomotor and exploratory behaviour in mice. *Research in Neuroscience*; 3(1): 7 – 21.

Osinubi, O, Onwuka, S, Olopade, J and Olude, A. (2019) Folic Acid Reverses the Effects of Cannabis on the Brain of New Born Wistar Rats. *Neuroscience and Medicine*; **10**: 213-223.

Oswald, Iain W. H.; Ojeda, Marcos A.; Pobanz, Ryan J.; Koby, Kevin A.; Buchanan, Anthony J.; Del Rosso, Josh; Guzman, Mario A.; Martin, Thomas J. (2021). "Identification of a New Family of Prenylated Volatile Sulfur Compounds in Cannabis Revealed by Comprehensive Two-Dimensional Gas Chromatography". *ACS Omega*. **6** (47): 31667–31676.

Podinić T, Werstuck G, Raha S (2023). The Implications of Cannabinoid-Induced Metabolic Dysregulation for Cellular Differentiation and Growth. *International Journal of Molecular Sciences*; 24(13):11003.

Pollastro, F.; Minassi, A.; Fresu, L.G. Cannabis phenolics and their bioactivities. *Curr. Med. Chem.* **2018**, 25, 1160–1185. [Google Scholar] [CrossRef] [PubMed]

Pope C, Mechoulam R, Parsons L (2010). Endocannabinoid signaling in neurotoxicity and neuroprotection. *Neurotoxicology*; 31(5):562–71.

Radwan, M.M.; ElSohly, M.A.; Slade, D.; Ahmed, S.A.; Wilson, L.; El-Alfy, A.T.; Khan, I.A.; Ross, S.A. Non-cannabinoid constituents from a high potency *Cannabis sativa* variety. *Phytochemistry* **2008**, 69, 2627–2633. [Google Scholar] [CrossRef][Green Version]

Ranganathan M, Carbuto M, Braley G, Elander J, Perry E, Pittman B, Radhakrishnan R, Sewell RA, D'Souza DC (2012). Naltrexone does not attenuate the effects of intravenous Δ^9 -

tetrahydrocannabinol in healthy humans. *International Journal of Neuropsychopharmacology*; 15:1251–64.

Regehr WG, Carey MR, Best AR. 2009. Activity-dependent regulation of synapses by retrograde messengers.

Neuron 63:154–170. DOI: <https://doi.org/10.1016/j.neuron.2009.06.021>, PMID: 19640475

Riboulet-Zemouli K (2020). "Cannabis' Ontologies I: Conceptual Issues with Cannabis and Cannabinoids terminology". *Drug Science, Policy and Law*; 6:1–37.

Rice, J. E., Vannucci, R. C. And Brierley, J. B. (1981). The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*; 9(2): 131 – 141

Riedel, G., Fadda, P., Mckillop-Smith, S., Pertwee, R. G., Platt, B. and Robinson, L. (2009). Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. *British Journal of Pharmacology*; 156(7): 1154 – 1166.

Rizzo MD, Crawford RB, Bach A, Sermet S, Amalfitano A, Kaminski NE (2019). Delta(9)-Tetrahydrocannabinol Suppresses Monocyte-Mediated Astrocyte Production of Monocyte Chemoattractant Protein 1 and Interleukin-6 in a Toll-Like Receptor 7-Stimulated Human Coculture. *Journal of Pharmacology and Experimental Therapeutics*; 371:191–201

Robin, L. M., Oliveira da Cruz, J. F., Langlais, V. C., Martin-Fernandez, M., Metna-Laurent, M., Busquets-Garcia, A., Bellocchio, L., SoriaGomez, E., Papouin, T., Varilh, M., Sherwood, M. W., Belluomo, I., Balcells, G., Matias, I., Bosier, B., Drago, F., van Eeckhaut, A., Smolders, I., Georges, F., ... Marsicano, G. (2018). Astroglial CB1 receptors determine synaptic D-serine availability to enable recognition memory. *Neuron*; 98: 935–944.

Rueda D, Galve-Roperh I, Haro A, Guzman M (2000). The CB(1) cannabinoid receptor is coupled to the activation of c-Jun N-terminal kinase. *Molecular Pharmacology*; 58:814–820.

Russo EB (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects". *British Journal of Pharmacology*; 163 (7): 1344–64.

Ryter SW, Cloonan SM, Choi AM (2013). Autophagy: a critical regulator of cellular metabolism and homeostasis. *Molecular Cell*; 36(1):7-16.

Saloner R. (2019) Neurocognitive Super Aging in Older Adults Living With HIV: Demographic, Neuromedical and Everyday Functioning Correlates. *Journal of the International Neuropsychological Society: JINS* 25:507–519.

Santuy, A., Tomás-Roca, L., Rodríguez, J.-R., González-Soriano, J., Zhu, F., Qiu, Z., (2020). Estimation of the number of synapses in the hippocampus and brain-wide by volume electron microscopy and genetic labeling. *Scientific Reports*; 10:14014. doi: 10.1038/s41598-020-70859-5

Sara Venturini, (2023). *The Cerebellum Structure-Position-Vasculature*. Revision 36.

Schaffner JH (1921-01-01). Influence of Environment on Sexual Expression in Hemp". *Botanical Gazette*; 71 (3): 197–219

Seibenhener, M. L. and Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments*; 2015(96): 52434.

Tait RJ, Mackinnon A, Christensen H (2011). Cannabis use and cognitive function: 8-year trajectory in a young adult cohort. *Addiction*; 106:2195–2203

Takagi M, Lubman DI, Cotton S, Fornito A, Baliz Y, Tucker A, Yucel M (2011). Executive control among adolescent inhalant and cannabis users. *Drug Alcohol Review*; 30:629–637

Takagi M, Yucel M, Cotton SM, Baliz Y, Tucker A, Elkins K, Lubman DI (2011). Verbal memory, learning, and executive functioning among adolescent inhalant and cannabis users. *Journal of Studies in Alcohol and Drugs*; 72:96–105

Takeda S, Ikeda E, Su S, Harada M, Okazaki H, Yoshioka Y, Nishimura H, Ishii H, Kakizoe K, Taniguchi A, Tokuyasu M, Himeno T, Watanabe K, Omiecinski CJ, Aramaki H (2014). Delta(9)-THC modulation of fatty acid 2-hydroxylase (FA2H) gene expression: possible involvement of induced levels of PPARalpha in MDA-MB-231 breast cancer cells. *Toxicology*; 326:18–24.

Tang Y, Le W (2016). Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases. *Molecular neurobiology*; 53:1181–1194

Tapert SF, Granholm E, Leedy NG, Brown SA (2002). Substance use and withdrawal: neuropsychological functioning over 8 years in youth. *Journal of International Neuropsychological Society*; 8:873–883.

Taura, F., Sirikantaramas, S., Shayama, Y and Morimoto, S. (2007). Phytocannabinoids in Cannabis Sativa: Recent Studies on Biosynthetic and Enzymes. *Chem. Biodiv.* 4 1649-1663.

Teichner G, Donohue B, Crum TA, Azrin NH, Golden CJ (2000). The relationship of neuropsychological functioning to measures of substance use in an adolescent drug abusing sample. *International Journal of Neuroscience*; 104:113–124.

Thames AD, Kuhn TP, Williamson TJ, Jones JD, Mahmood Z, Hammond A (2017). Marijuana effects on changes in brain structure and cognitive function among HIV+ and HIV–adults. *Drug Alcohol Depend*; 170:120–127.

Theodosis, D. T., Poulain, D. A., and Oliet, S. H. R. (2008). Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiology Reviews*; 88:983–1008.

Theunissen EL, Heckman P, de Sousa Fernandes Perna EB, Kuypers KPC, Sambeth A, Blokland A, Prickaerts J, Toennes SW, Ramaekers JG (2015). Rivastigmine but not vardenafil reverses cannabis-induced impairment of verbal memory in healthy humans. *Psychopharmacology (Berl)*; 232:343–53.

Töpperwien, M., van der Meer, F., Stadelmann, C., and Salditt, T. (2020). Correlative x-ray phase-contrast tomography and histology of human brain tissue affected by Alzheimer's disease. *NeuroImage*; 210:116523.

Toson ESA (2011). Impact of marijuana smoking on liver and sex hormones: Correlation with oxidative stress. *Nature and Science*; 9(12):76- 87. 32.

Tremblay, M. -È, Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biology*; 8:e1000527.

Tsuru-Aoyagi, K., Potts, M. B., Trivedi, A., Pfankuch, T., Raber, J., Wendland, M., Claus, C. P., Koh, S. E., Ferriero, D. and Noble-Haeusslein, L. J. (2009). Glutathione peroxidase activity modulates recovery in the injured immature brain. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*; 65(5): 540 – 549.

Varvel SA, Lichtman AH. 2002. Evaluation of CB1 receptor knockout mice in the morris water maze. *The Journal*

of Pharmacology and Experimental Therapeutics 301:915–924. DOI: <https://doi.org/10.1124/jpet.301.3.915>, PMID: 12023519

Vella RK, Jackson DJ, Fenning AS (2017). Δ^9 -Tetrahydrocannabinol prevents cardiovascular dysfunction in STZ-diabetic Wistar-Kyoto rats. *Biomedical Research International*; 2017:7974149.

Verkhatsky, A., and Nedergaard, M. (2018). Physiology of astroglia. *Physiological Review*; 98, 239–389.

Vrechi TAM, Leão AHFF, Morais IBM, Abílio VC, Zuardi AW, Hallak JEC, Crippa JA, Bincotto C, Ureshino RP, Smaili SS, Pereira GJS (2021). Cannabidiol induces autophagy via ERK1/2 activation in neural cells. *Scientific Reports*; 8;11(1):5434.

Wang, Y., Branicky, R., Noë, A. and Hekimi, S. (2018). Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. *Journal of Cell Biology*; 217(6): 1915 – 1928.

Watson CW, Paolillo EW, Morgan EE, Umlauf A, Sundermann EE, Ellis RJ, Letendre S, Marcotte TD, Heaton RK, Grant I (2020). Cannabis Exposure is Associated With a Lower Likelihood of Neurocognitive Impairment in People Living With HIV. *Journal of Acquired Immune Deficiency Syndrome*; 83:56–64

Wu G, Fang Y-Z, Yang S, Lupton JR, Turner ND (2004). Glutathione metabolism and its implications for health. *Journal of Nutrition*; 134(3):489 –92.

Xu J, Chavis JA, Racke MK, Drew PD (2006). Peroxisome proliferator-activated receptor-alpha and retinoid X receptor agonists inhibit inflammatory responses of astrocytes. *Journal of Neuroimmunology*; 176:95–105.

Xu P, Wang Y, Qin Z, Qiu L, Zhang M, Huang Y, Zheng JC (2017) Combined Medication of Antiretroviral Drugs Tenofovir Disoproxil Fumarate, Emtricitabine, and Raltegravir Reduces Neural Progenitor Cell Proliferation In Vivo and In Vitro. *J Neuroimmune Pharmacol* 12:682– 692

Yinka OS, Olubunmi OP, Zabdiel AA, Oladele OJ, Taiye AS, Ayodele A, Adetutu FO, Afees OJ, Kayode AA (2023). Peroral Exposure to *Cannabis Sativa* Ethanol Extract Caused Neuronal Degeneration and Astroglialosis in Wistar Rats' Prefrontal Cortex. *Annals of Neuroscience*; 30(2):84-95.

De Vita, S.; Finamore, C.; Chini, M.G.; Saviano, G.; De Felice, V.; De Marino, S.; Lauro, G.; Casapullo, A.; Fantasma, F.; Trombetta, F.; et al. Phytochemical Analysis of the Methanolic Extract and Essential Oil from Leaves of Industrial Hemp Futura 75 Cultivar: Isolation of a New Cannabinoid Derivative and

Biological Profile Using Computational Approaches. *Plants* **2022**, *11*, 1671. <https://doi.org/10.3390/plants11131671> Academic Editor: Ain Raal Received: 3

Muscarà, C., Smeriglio, A., Trombetta, D., Mandalari, G., La Camera, E., Grassi, G., & Circosta, C. (2021). Phytochemical characterization and biological properties of two standardized extracts from a non-psychoactive *Cannabis sativa* L. cannabidiol (CBD)-chemotype. *Phytotherapy Research*, *35*(9), 5269–5281. <https://doi.org/10.1002/ptr.7201>

Pino, S.; Espinoza, L.; Jara-Gutiérrez, C.; Villena, J.; Olea, A.F.; Díaz, K. Study of Cannabis Oils Obtained from Three Varieties of *C. sativa* and by Two Different Extraction Methods: Phytochemical Characterization and Biological Activities. *Plants* **2023**, *12*, 1772. <https://doi.org/10.3390/plants12091772>

Smith CJ, Vergara D, Keegan B, Jikomes N (2022) The phytochemical diversity of commercial *Cannabis* in the United States. *PLoS ONE* 17(5): e0267498. <https://doi.org/10.1371/journal.pone.0267498>.

Mazzara, E.; Torresi, J.; Fico, G.; Papini, A.; Kulbaka, N.; Dall'Acqua, S.; Sut, S.; Garzoli, S.; Mustafa, A.M.; Cappellacci, L.; et al. A Comprehensive Phytochemical Analysis of Terpenes, Polyphenols and Cannabinoids, and Micromorphological Characterization of 9 Commercial Varieties of *Cannabis sativa* L. *Plants* **2022**, *11*, 891. <https://doi.org/10.3390/plants11070891>

Sinha AK. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*. 47: 389-394.

Yadav-Samudrala BJ, Gorman BL, Barmada KM, Ravula HP, Hugueley CJ, Wallace ED, Peace MR, Poklis JL, Jiang W and Fitting S. (2024) Effects of acute cannabidiol on behavior and the endocannabinoid system in HIV-1 Tat transgenic female and male mice. *Front. Neurosci.* 18:1358555. doi: 10.3389/fnins.2024.1358555

Osinubi, O.O., Onwuka, S.K., Olopade, J.O. and Olude, A.M. (2019) Folic Acid Reverses the Effects of Cannabis on the Brain of New Born Wistar Rats. *Neuroscience & Medicine*, 10, 213-223. <https://doi.org/10.4236/nm.2019.103016>