Original Research Article

EFFECT OF AQUEOUS EXTRACT OF *CANNABISSATIVA* LEAF ON THE OXIDATIVE STRESS MARKERS IN THE BRAIN OF 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 28days 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 28days 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 administration of cannabis sativa induced oxidative stress in a dose dependent fashion.It can therefore be concluded that there were dose and time dependent toxic effects of cannabis Sativa in the model animals.

Keywords: Cannabis sativa; oxidative stress; malondialdehyde; superoxide dismutase; catalase

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).

The Effects of Cannabis Sativa on the Oxidative Stress Markers of the Brain

Oxidative stress is a common feature of acute or chronic neurodegenerative diseases like Alzheimer's disease, multiple sclerosis, Parkinson's disease, and amyotrophic lateral sclerosis (Pacher *et al*., 2007). Oxidative stress also provides a key link between environmental factors (e.g., heavy metals, pesticides, and herbicides) with genetic risk and endogenous factors in the pathogenic mechanisms of neurodegeneration. Disruption of the blood-brain barrier's integrity and reactive changes in the glial elements in the CNS, which facilitates the penetration of inflammatory cells and various toxins to the site of brain injury and leads to irreversible degeneration, are caused by oxidative stress (Pacher *et al*., 2007). Moreover, various forms of acute (e.g., stroke, traumatic brain injury, and epilepsy) or chronic (e.g., Alzheimer's disease, multiple sclerosis, Huntington's disease, Parkinson's disease, HIV associated dementia, etc.) neurodegenerative disorders are caused by

dysregulation of the endocannabinoid system (Bisogno & Di Marzo, 2010), as reflected by the increase or decrease in endocannabinoid content or altered CBRs expression in diseased animal or human tissues. Interestingly, the neuroprotective potentials of plant-derived cannabinoids in the CNS have been established (Pope *et al*., 2010), and this is mediated via their antioxidant property among others (Pacher & Haskó, 2008).

Abdulrahim *et al*., (2021) in their study observed that the CS increased G6PD, GPx, and SOD, but decreased NO and had no effect on MDA, CAT, GR, and AChE. Their data support the contention that CS elicits an anti-oxidative effect on the brain tissue. They also speculate that the increase in the G6PD (which is a second line anti-oxidant) in the brain of rats that received CS + M was a reactive response to the depletion in the first-line anti-oxidants (SOD and GPx). The SOD, a ubiquitous metal-containing enzyme, converts superoxide anion into O_2 and H_2O_2 (Çimen, 2008). The GPx is an enzyme family with peroxidase that protects organisms from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (Muthukumar *et al*., 2011). Reduced glutathione (GSH), which is a tripeptide molecule that consists of L-glutamate, L-cysteine, and L-glycine, is the most important antioxidant and free radical scavenger in the brain. In the presence of GPx, the GSH is oxidized (via removal of hydrogen) by hydrogen peroxide to form oxidized glutathione disulfide (GSSG), which can also be converted back to GSH by glutathione reductase (Bhabak& Mugesh, 2010). Therefore, the ratio of reduced (GSH)/oxidized (GSSH) determines the redox state of cells (Wu *et al*., 2004). Their observation of an increase in the brain SOD and GPx in their study and the previously reported increase in the brain GSH (Abdel-Salam *et al*., 2018) in rats suggest an antioxidative potential of both the ethanol and chloroform extracts of CS respectively.

Pathological changes in the brain cause a rapid alteration in the morphology and phagocytes behavior of microglial cells, leading to an increase in their cytotoxic responses characterized by secretion of NO, proteases, and cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-1β. An increase in neuronal NO has been implicated in the endoplasmic reticulum stress and peroxynitrite-mediated oxidative/nitrosative damage (Zhu *et al*., 2017). Our observation of a decrease in NO without a change in MDA contrasts the previously reported increase in the MDA but unchanged NO in the brain of pentylenetetrazole-treated rats (Abdel-Salam *et al*., 2018). While their study suggested that CS causes lipid peroxidation even when GSH increases, our present study suggests that CS reduces nitrosative stress by reducing NO. The reduction in NO and the corresponding increase in antioxidant enzymes (e.g., G6PD, GPx, and SOD) by CS observed in our study might be responsible for the absence of lipid peroxidation (evident from the unchanged level of MDA) in CS-treated rats. These corroborate the contention that CS is neuroprotective especially by enhancing antioxidants and suppressing oxidative and nitrosative stresses.Bhunia *et al*, 2022 reported a non-psychoactive phytocannabinoid CBD holds significant promises due to their antioxidant and anti-inflammatory properties, high safety profile, and good tolerability (Scholey *et al*., 2009). Though there were no significant changes in the levels of activity of glutathione peroxidase in the treatment groups except in the 1 % cannabis-diet, where the increases

observed may mediate a reduction in oxidative stress in mice brain due to the effects of Cannabis or its constituents. This may be possible by the initiation of repair to traumatically injured brain tissues as previously demonstrated in transgenic mice (Tsuru-Aoyagi *et al*., 2009). In contrast, the levels of superoxide dismutase in animals that consumed Cannabis were significantly lower relative to control mice. The significant loss of superoxide dismutase activity is a manifestation of oxidative damage of the brain (Wang *et al*., 2018). Likewise, there was a dose-dependent decline in the levels of malondialdehyde in the cannabis diet groups, another indication of the oxidative damage potential of Cannabis consumed orally in this study. Interestingly, the changes recorded in the different antioxidant biomarkers assayed in brain tissues of the study animals did not alter to any noticeable extent the behaviour of the mice.

A trade-off between the generation of oxidative radicals and oxidative defence mechanisms in the brain may have been elicited by different constituents of Cannabis. There were no correlations between the mild changes in behavioral patterns and oxidative stress differentials in mice that consumed Cannabis within study period (Akinola *et al*, 2019). Abdel-Salam *et al* (2020).We assessed lipid peroxidation in both the brain homogenates and serum by measuring the level of malondialdehyde. The latter is an end product of polyunsaturated fatty acid peroxidation which indicates free radical attack on their side chain and is therefore a marker of membrane lipid peroxidation (Gutteridge, 1995).When treating rats with only cannabis extracts, there was marked increase in the level of malondialdehyde in serum in contrast to nonsignificant increase in brain tissue (McGilveray, 2005). It was also noted that the increments in serum malondialdehyde due to the cannabis resin were more evident at the doses of 5 or 10 mg/kg compared with the higher dose of 20 mg/kg. It is also possible that stimulation of compensatory/ antioxidant mechanisms accounts for this observation Bloomer *et al*., (2018).Other studies in rodents using marijuana or cannabis resin extracts failed to demonstrate increased lipid peroxidation in the brain and even a moderate though a significant decrease in lipid peroxidation was observed with the cannabis being given at 20 mg/kg (Abdel-Salam *et al*., 2013; Abdel-Salam *et al*., 2014). A likely explanation for this observed discrepancy between the effect of resin-based and marijuana-based extracts is the resin and marijuana relative content of different cannabinoids, the presence of flavonoids in fresh leaves of marijuana, possible resin additives and/or longer administration time of the extract in the present study. On the other hand, increased lipid peroxidation was detected in brain and serum after treatment with tramadol, while the higher dose of the drug caused significant decrease in reduced glutathione. These findings are consistent with other studies in the rat (Awadalla Salah-Eldi, 2016; Abdel-Salam *et al*., 2019). Our present findings in addition indicate that the higher dose of cannabis resin was able to increase serum level of reduced glutathione by 46% relative to the vehicle control value. Several previous studies have shown increased brain reduced glutathione by cannabis administration in rodents. Cannabis has also been shown to increase brain catalase activity (Abdel-Salam., 2014), superoxide dismutase activity and ascorbic acid content (El-Hiny*et al*., 2019) in rat brain. Other researchers found no effect for THC on erythrocyte GSH, plasma catalase or superoxide dismutase in normal rats. In diabetic rats, however, treatment with THC increased erythrocyte GSH and plasma superoxide dismutase activity (Coskun and Bolkent, 2016). Cannabis

thus exerts a complex action with both antioxidant and prooxidant effects being reported as mentioned above.

In a study of the combined and independent effects of chronic cannabis use and HIV on brain metabolites, Chang *et al*. (2006) found that cannabis use was associated with a decrease in neuronal and glial metabolites, yet a normalization of glutamate levels in PWH (Chang *et al*., 2006). **Kubiliene*et al*., 2021** 51 reported significant increase in hepatic catalase activity in male treated withcannabis sativa extract 20 min before the exposure of various oxidants. Kubiliene et al.,2021 observed no significant changes in GSH levels in blood or MDA levels in the brain and liver of experimental mice after exposure to Cannabis sativa L. extract. However, it significantly decreased these concentrations after AlCl3-induced oxidative stress and reached the same concentration as in the vehicle group. CAT activity is considered to be a sensitive biomarker of oxidative stress (Atli et al.,2006) A decrease in catalase activity in cells indicates a state of oxidative stress in the cell. Treatment with Cannabis sativa L. extract alone showed a significant difference in CAT activity in the liver and brain of the experimental mice compared to that in the control group (Kubiliene et al.,2021). Kubiliene et al.,2021 results also suggest that Cannabis extract may regulate oxidative stress as it has a significant role in increasing the activity of CAT in plasma. This is consistent with the results of other studies showing the ability of hemp seed peptides to increase CAT activity in plasma (Hammoud & Shalaby,2019). Kubiliene et al., (2021) results demonstrated that Cannabis sativa L. extract significantly altered GSH and MDA concentrations as well as CAT activity associated with AlCl3-induced toxicity.

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 28days; **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 homogenized in phosphate buffer solution at 10,000rpm. It was later centrifuge to separate the supernatant from the residue. The supernatant was used for the oxidative stress parameters analysis.

Oxidative Stress Analysis

Malondialdehyde (MDA) was evaluated by colorimetric method of Gutteridge and Wilkins, (1982). Catalase was determined by colorimetric method of Sinha, (1972). Superoxide dismutase (SOD) was determined by the colorimetric method of (Friedewaldand Fredovich, 1972).

Statistical Analysis

The data were presented as Mean ± 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

Results are presented as Mean ± SD of 5 rate in each group $p< 0.05$ is considered statistically significant. The result presented in table 1 above shows no statistically significant difference in serum malondialdehyde (MDA) levels of rat on the experimental groups B, C, D and E compared to control group A.

TABLE 1: RESULT OF SERUM MALONDIALDEHYDE

Group	MDA	P-value
A	2.05 ± 0.26	

B	2.28 ± 0.28	0.211
C	2.32 ± 0.58	0.380
D	2.16 ± 028	0.537
E	2.09 ± 0.23	0.803

The result of serum catalase level shows that catalase levels were significantly reduced in the experimental groups B, C, D and E compared to the control group A.

This study shows MDA present no significant difference on serum level on the model groups when compared to control group but CAT and SOD present significant reduction in serum level reason. Abdulrahim et.al (2021) in their study observed that cannabis Sativa had no effect on MDA which is in consistent with this study. This data supports the contention that cannabis sativa elicits an anti-oxidative effect on the brain. In these studies, we observed no significant difference in MDA, which is similar with what Abdul-Salam and colleagues reported, this indicate that cannabis sativa reduces nitrosative stress by reducing No (Nitric oxide). Similarly, Bloomer et.al, (2018) in their study on young and physically active subjects, however found no significant difference in serum malondialdehyde or advanced oxidation protein produces between marijuana smokers and non-smokers.

In this study there was a significant reduction in CAT and SOD, this is consistent with Abdulrahim et.al (2021) who also observed that M increased G6PD but reduced SOD in the brain of CS treated rats. They speculated that the increases in the G6PD (which is a second line anti-oxidant) in the brain of rats that received CS+M was a reactive response to the depletion in the first line anti-oxidant (SOD). Kubiliene et.al (2021) observed no significant changes in serum MDA level of experimental mice after exposure to cannabis Sativa leave extract, this study also reported similar result of no significant difference of MDA level of rat on the experimental group as when compared to the control.

CAT activity is considered to be a sensitive biomarker of oxidative stress. This study reports a significant decrease in CAT which comes to terms with Atli et.al (2006). A decrease in catalase activity in cell indicate a state of oxidative stress in the cell.

CONCLUSION

Conclusively, it could therefore be deduced that there were but dose and time dependent toxic effects of cannabis Sativa in the model animals. There was increased in appetite which lead to weight gain, attesting that endocannabinoid in the hypothalamus activate cannabinoid receptors that are responsible for maintaining food intake, thereby increasing body weight in human and animals. Cannabis Sativa was showed to cause marked neuronal aberrations in the cerebellum of Wistar rats with changes in GFAP immunoreactivity especially in severely exposed model. Furthermore, the reasons for the disparities of

phytochemical screening test may be due to differences in plant species or in topography of plant. This finding indicates that exposure to delta – 9 THC, the psychoactive ingredient of cannabis Sativa at doses commensurate with those used by human cannabis Sativa users can produce cerebellum alteration, function and structures.

Recommendation

It is recommended that more studies can be done comparing the impact of cannabis on the investigated parameters for shorter and longer durations and also with lower and higher doses

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 HIVinfected 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, lacono 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 Δ9-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 cannabisinduced 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 Journalof 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). Δ9 -Tetrahydrocannabinol prevents cardiovascular dysfunction in STZ-diabetic Wistar-Kyoto rats. *Biomedical Research International*; 2017:7974149.

Verkhratsky, 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, Bincoletto 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 Astrogliosis 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-psychotropic 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 BiologicalcActivities. Plants **2023**, 12, 1772.chttps://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, Huguely 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