

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 A received distilled water for 28 days. Group B, C, D and E served as the low, high, low dose recovery and high dose recovery group respectively. Group B were administered with 10mg/kg body weight of cannabis sativa leave aqueous extract for 28 days. Group C were administered with 20mg/kg body weight of cannabis sativa leave aqueous extract for 28 days. Group D 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 E received 20mg/kg body weight of cannabis sativa for 28 days and were allowed for further 28days without administration. GroupsD and E represent the recovery groups. Group A, B and C were sacrificed a day after their last intubation. The result of the study showed that administration of cannabis sativa led to a non-significant increase in MDA and a corresponding significant reduction in SOD and CAT levels in the experimental groups compared to the control group A. This is a pointer to the presence oxidative stress. 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

The high content of psychoactive compounds in Cannabis sativa leads to its common abuse (Lucas et al., 2018). The medical use of cannabis is attributed to its antioxidant, anticonvulsant, anti-

inflammatory, and neuroprotective properties, but its adverse effects should not be taken lightly (Ford et al., 2017 ; da Silva et al., 2018).

Cannabis sativa is an annual herbaceous flowering plant that originated in Eastern Asia and now has a worldwide distribution due to its widespread cultivation. It has been grown throughout the course of history, utilized for industrial fiber, seed oil, food, recreation, religious and spiritual practices, and medicinal purposes. Harvesting each part of the plant is done differently, depending on the purpose of its use. *Cannabis sativa* has flowers that bloom during short daylight hours, with staminate (male) plants being generally taller and less sturdy than pistillate (female) plants (United Cannabis Seeds 2021). The flowers of the female plant are grouped into racemes and can yield several seeds. The pollen from male plants is shed and dies a few weeks before the seeds ripen on female plants. Heritable X and Y chromosomes ensure that both sexes are produced in equal numbers when light is present for 12 to 14 hours under typical conditions (Clark and Merlin, 2013). Even though genetic factors are the main factor in determining whether a plant becomes male or female, environmental factors, like the diurnal light cycle, can have an impact on sexual expression (Schaffner, 2020).

Safety considerations hinge on comprehending potential toxicity, particularly when the plant extract is utilized in traditional medicine or as a dietary supplement. The use of cannabis is prevalent among teens and young adults, but the long-term ramifications of doing so are a matter of contention. The onset of cannabis consumption generally occurs during early adolescence and increases in the mid-20s (Hasin et al. 2015). According to Azofeifa 2016, a survey conducted in the United States found that 7.4% of teenagers had reported using cannabis in the past month and 13.1% had done so in the past year. The use of cannabis can lead to negative health impacts, such as increased chances of developing lung, cardiovascular, and periodontal diseases (Gordon et al. 2013; Jouanjus et al. 2017). There has been a lack of conclusive evidence about its influence on the development of cognitive and affective dysfunction. An initial study indicated that cannabis usage, particularly while in adolescence, leads to a lasting decline in neurocognition, which can result in an 8-point drop in IQ between childhood and adulthood (Jackson et al.). 2016). However, this conclusion is not supported by recent studies. Cannabis users, for instance, exhibit a lower performance on cognitive tests than non-users, but their scores are comparable to their non-using twins (Meier et al). 2018). The brain is home to receptors for

THC and other cannabinoid compounds, with concentrations in the frontal cortex, basal ganglia, cerebellum, and limbic regions. Cannabinoid activity in the basal ganglia and cerebellum is believed to be responsible for the influence on psychomotor control (John, 2003). Monitoring and refining ongoing movements can be achieved through sensorymotor signals, while changes in behavioral state, such as arousal and locomotor activity, have an impact on sensory processing and perception (McGinley, Schneider, and Mooney, 2015; Vinck, et al., 2015; Pakan, et al., 2016). Locomotor activity and arousal are implicated in modulating delayed eyeblink conditioning, which is a form of associative learning that involves the cerebellum (Albergaria et al., 2018).

Behavioral state across species is profoundly influenced by cannabinoids (Mackie, 2007; Oakes et al., 2017; Luchtenburg et al., 2019). In a short period of time, cannabis and THC have a variety of effects on various 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) reported that D9-tetrahydrocannabinol, which is responsible for the psychoactive properties of cannabis, causes autophagy to be disrupted specifically in the striatum, which is responsible for controlling motor behavior, both in vitro and in vivo. In mice, D 9-tetrahydrocannabinol-related impairment of motor coordination can be rehabilitated by increasing autophagy by either pharmacologically (with temsirolimus) or dietary intervention (with trehalose). These findings indicate that inhibition of autophagy is a unique mechanistic link between cannabinoid use and motor performance. Additionally, activators of autophagy could be utilized as potential therapeutic tools to address specific behavioral changes caused by cannabinoid use.

The influence of cannabis use on decision-making, particularly when it comes to taking risks, is a matter of concern. Differentiation between cannabis users and non-users has been observed by self-report questionnaires and laboratory risk-taking tasks (Burggren et al, 2019). Neurocognitive performance, macrostructural and microstructural brain development, and alterations in brain functioning are frequently exhibited by adolescents and teens who engage in heavy marijuana use. It is unclear if these disadvantages are due to differences that have already been present, leading to an increase in substance use and more 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). The development of cognitive functions in memory and executive functioning, particularly in specialized functions such as cognitive control, is not only closely linked to adolescence and neocortical tissue maturation, but it also has potential to impact school performance and participation in risk/reward behaviors (Casey et al,2008).

Schwartz et al. (1989) conducted a study that first assessed the effects of marijuana on adolescent neurocognitive development and evaluated verbal and nonverbal memory performance of cannabis-dependent adolescents (ages 14 to 16) compared to controls. Schwartz and colleagues found that monitored abstinence for six weeks did not prevent short-term memory impairment. Teichner and colleagues (2000) found no correlation between the severity of marijuana use and cognitive performance among adolescents with and without cognitive impairment referred for drug treatment.

According to Takagi and colleagues, cannabis users (ages 13-24) did not perform as well on measures of immediate and delayed verbal memory compared to community controls. No discrepancies were observed between cannabis users and community controls on measures of executive functioning in a study conducted by this team of investigators (Takagi et al., 2011). Similarly, Gonzalez and colleagues (2012) found differences in immediate and delayed recall among young adult cannabis users (approximately age 20) compared to non-using controls, but no differences were observed in measures of impulsivity. Even though there were no group differences in impulsivity, the authors found that poor performance on a decision-making task was linked to increased symptoms of cannabis use disorder. The study by Solowij and colleagues examined 181 adolescents (ages 16–20) and discovered that cannabis users performed worse on learning and recall, and the worsening performance was linked to the 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). A group of researchers has suggested that a lifelong history of cannabis use disorders decreases the likelihood of neurocognitive impairment in patients with Parkinson's disease (Watson et al., 2020) and may even lead to more youthful and resilient neurocognitive abilities among adults aging with HIV (Saloner et al., 2019b).

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 25 adult Wistar Rats weighing between 180g-200g was used for this study. The rats were separated into 5 groups (A,B,C,D &E) with 5 rats in each 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 Friedewald and Fredovich (1972).

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

Results are presented as Mean \pm 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 \pm 0.26	
B	2.28 \pm 0.28	0.211
C	2.32 \pm 0.58	0.380
D	2.16 \pm 0.28	0.537
E	2.09 \pm 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.

TABLE 2: RESULT OF SUPEROXIDE DISMUTASE (SOD)

GROUP	SOD	P-VALUE
A	25.03 \pm 1.64	
B	22.92 \pm 1.12	0.045
C	22.13 \pm 2.42	0.057
D	22.10 \pm 2.15	0.042
E	20.40 \pm 2.34	0.007

The result of SOD presented in table 3 above shows significant reduction in SOD levels in the experimental groups B, D and E compared to the control group A

TABLE 3: RESULT OF SERUM CATALASE LEVEL

Group	CAT	P-VALUE
A	34.46 ± 1.90	
B	30.19 ± 1.80	0.006
C	28.17 ± 3.01	0.004
D	29.75 ± 2.50	0.010
E	29.67 ± 1.10	0.001

F – Value $F(4,20) = 6.00$ $P < 0.0024$

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 this study, we observed no significant difference in MDA, which is similar with what Abdul-Salam and colleagues reported. Similarly, Bloomer et.al, (2018) in their study on young and physically active human subjects 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 reduced SOD levels in the brain of rats exposed to cannabis sativa. They speculated that the increases in the G6PD (which is a second line anti-oxidant) in the brain of rats that received CS 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 corroborates the findings of the present study.

CAT activity is considered to be a sensitive biomarker of oxidative stress. This study reports a significant decrease in CAT which is in line with the report of Atli et.al (2006). A decrease in catalase activity in cells indicate a state of oxidative stress subject to indications from other parameters.

CONCLUSION

It could be deduced from the result of this study that there were dose and time dependent toxic effects of cannabis Sativa in the exposed animals. There significant weight gain, attesting that endocannabinoid may activate cannabinoid receptors that are responsible for increasing food intake, thereby increasing body weight in rats. Cannabis Sativa was shown to cause marked neuronal alterations in the cerebellum of Wistar rats. This finding may also infer that exposure to delta – 9 THC, the psychoactive ingredient of cannabis Sativa at the doses used in this study can produce cytoarchitectural distortion in the cerebellum of Wistar rats.

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 O. 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, 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-Haesslein, 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 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 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>

Mathieu, M. E. J., Lucie, O., Omam, Elisabeth, N. B., Achick, T. E., Roger, A., Hermann, C., Nina, N., Pamela, T., Marie-Claire, F., Wilfred, M., & Charles, F. (2024). Evaluation of the Antioxidant Capacity and Studies of the Anticonvulsant Properties of *Hymenocardia acida*. *Journal of Advances in Biology & Biotechnology*, 27(3), 129–139. <https://doi.org/10.9734/jabb/2024/v27i3728>

Okon, A. J., Aluko, O. O., Okon, I. J., Inah, S. A., Dania, A. V., & Achi, F. I.-ogim. (2023). Effect of *Carica papaya* Leaves and Seeds on Electrolyte and Hematological Parameters in Albino Wistar Rats Exposed to Lead Nitrate. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 13(4), 15–27. <https://doi.org/10.9734/ajbgmb/2023/v13i4300>

Manoj Kumar V, Henley AK, Nelson CJ, Indumati O, Prabhakara Rao Y, Rajanna S, Rajanna B. Protective effect of *Allium sativum* (garlic) aqueous extract against lead-induced oxidative stress in the rat brain, liver, and kidney. *Environmental Science and Pollution Research*. 2017 Jan;24:1544-52.