The Neurotoxic Effects of Cannabis on Brain: Review of Clinical and Experimental Data

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Abstract: - Cannabis is the most widely used illicit drug worldwide. Evidence indicated negative impact for cannabis on the brain. Animal research and in vitro studies using delta-9-THC (THC) or cannabis extracts with high THC content provided evidence for a detrimental effect on neuronal integrity with DNA damage, cell shrinkage, atrophy and apoptosis. The mechanisms by which herbal cannabis affects brain structure and function are not clear but impaired mitochondrial functioning, reduced glucose availability and inhibition of brain energetic metabolism by cannabis have been shown. Clinical studies investigating the effects of cannabis in humans found raised serum levels of proinflammatory cytokines in chronic cannabis users. Human studies also indicated increased oxidative stress biomarkers and reduced antioxidants in blood of chronic cannabis users. Preclinical data on the effect of cannabis or THC on oxidative stress, however, were less conclusive in that cannabis might increase or attenuate oxidative stress and neurotoxicity. The aim of this review is to summarize the evidence from animal and clinical studies pertaining to the toxic effects of cannabis and its main psychoactive ingredient THC on the brain and possible mechanisms involved.

Key-Words: - delta-9-tetrahydrocannabinol; brain; cannabis; oxidative stress; neuroinflammation

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1 Introduction

The psychoactive plant Cannabis sativa is the most widely used illicit drug worldwide, with an estimated 192 million people having used cannabis in 2018. Cannabis use is also on the rise with the increasing legalisation for medicinal and recreational use [1]. The term ‘marijuana’ or ‘marihuana’ refers to the flowering tops and leaves of the female plant while the resin obtained from these flowers is known as ‘hashish’ [2]. The chemistry of *Cannabis sativa* plant is complex with over 650 compounds and a group of a terpenophenolic compounds, unique to cannabis and known as cannabinoids [2,3]. To date, more than 120 cannabinoids have been described [4], the most abundant of them is the Δ^9-tetrahydrocannabinol (THC) which is responsible for the psychomimetic effects that occurs when cannabis is smoked such as feelings of euphoria, distortion of time perception, altered sensory experiences, motor incoordination and impairments of memory [5,6]. Cannabidiol (CBD) is another cannabinoid present in small mounts in the cannabis plant, but is devoid of psychotropic effects and appears to antagonize some of the biological effects of THC. The latter exerts its effects by acting on two types of G-protein coupled receptors; cannabinoid CB1 and CB2 receptors [7]. CB1 receptors are abundant in brain areas associated with cognition, memory, learning, emotions, appetite, and motor coordination and mediate most of the central actions of cannabis in humans. CB2 receptors are mainly found on cells of the immune system in the periphery. There are also endogenous compounds, the endocannabinoids that can act on cannabinoid receptors eg., anandamide and 2-arachidonylglycerol [8].

The last decade has witnessed increasingly growing interest in using cannabis for treating a variety of medical conditions.. The inhalation of THC-containing cannabis was reported by patients with Parkinson’s disease [9], fibromyalgia [10], sickle cell disease [11], inflammatory bowel disease [12], neuropathic pain [13]. Formulations of whole plant extract containing 1:1 THC/CBD in the form of an oromucosal spray is used by patients with multiple sclerosis for improving spasticity [14] and bladder dysfunction [15].

There is evidence, however, that indicate a negative impact for cannabis on the brain on the short- and on the long-term [16,17]. Adolescents and young adults are particularly prone to the health hazards of cannabis with decline in IQ among cannabis users, impaired cognitive performance, lower academic

achievement and educational outcome with longer time to graduation [18,19]. Cannabis induces impairment of driving performance increasing road accidents [20]. Evidence links cannabis use to development of psychotic events late in life [21].Users of cannabis might also suffer ischaemic stroke, more frequently in young men [22]. Neuroimaging studies of cannabis's influence on brain in humans have shown structural alterations indicative of neurotoxic effects [23]. The present review therefore aims to shed light on the consequences of cannabis use and its main psychoactive compound THC on brain neuronal integrity and the underlying mechanisms involved in neurotoxicity.

2 Evidence that Cannabis is Toxic to Cells

Animal research and in vitro studies using cannabis extracts rich in THC or its main psychotropic ingredient THC provided strong evidence for a detrimental effect for THC on neuronal integrity [24-28]. Sarafian et al. [25] found that exposing endothelial cells to smoke from marijuana cigarettes for 30 min induced a time-dependent increase in necrotic cell death that increased steadily reaching reached 78% at 48 h. In vitro studies using A549 cells, a human lung cancer cell line, showed that THC decreased in ATP levels with an IC50 value of 7.5 μg/ml, decreased mitochondrial membrane potential (at concentrations of 2.5 or 10 μg/ml) as early as 1 h after exposure and caused cell death [26]. Zhu et al. [29] showed that treatment of murine ConA-activated splenocytes and LPS-activated peritoneal macrophages in culture with THC (10 μg/ml) increased DNA fragmentation. THC treatment decreased Bcl-2 mRNA and protein in splenocytes. THC–induced apoptosis was blocked by the caspase-1 inhibitor. Downer et al. [30,31] reported that THC-induced apoptosis of primary rat cortical neurons. The mechanism involved the activation of c-jun N terminal kinase, release of cytochrome c, activation of caspase-3, an increase in Bax expression, and cleavage of the DNA repair enzyme poly-ADP ribose polymerase. The THC-induced apoptosis was blocked by the CB (1) receptor antagonist AM-251. Gowran and Campbell [32] showed in addition that in primary cortical neurons, THC (5 μM) caused rapid, but transient, increase in lysosomal membrane permeability. This effect on lysosomal integrity occurred within 15 min after exposure to THC and was maintained for 30 min. THC-induced caspase-3 activation and apoptotic cell death by evoking the release of the lysosomal cathepsin enzyme, cathepsin-D, into the cytosol. These effects of THC were CB-mediated and involved the tumour suppressor protein, p53.

Chan et al. [24] found that concentrations of THC as low as 0.5 μM were toxic to rat hippocampal neurons. Rat hippocampal slices were exposed to THC. The CA1 pyramidal cell layer of hippocampal slices exposed to THC exhibited condensed, contracted and smaller nuclei, shrinkage of neuronal cell bodies and genomic DNA breakage. Steel et al. [27] reported impaired hippocampal neuroplastic response, and new-born neurons induced by training in rats after intraperitoneal (i.p) injection of THC (6 mg/kg).

Studies in rats treated with THC (10 or 20 mg/kg orally) for 90 days revealed reduction in the dendritic length of CA3 pyramidal neurons. The higher dose of 60 mg/kg induced 44% reduction in the number of synapses per unit volume [33]. Landfield et al. [34] found decreased neuronal density and increased glial cell reactivity in the hippocampus of rats treated chronically THC for 8 months. Other studies investigated the effect of cannabis extracts containing 10%THC in vivo. Rats received cannabis extracts at doses of 5, 10 or 20 mg/kg (expressed as THC), i.p., once a day for 30 days. Brain sections showed the presence of dark neurons with small or undefined nuclei, cellular infiltration, and gliosis. Immunohistochemical staining with caspase 3 antibody, revealed increased number of positively reactive cells, indicative of apoptosis. Electron microscopy of a neuron from rats treated with 20 mg/kg cannabis, showed an elongated nucleus, discontinued nuclear envelope and dispersed rough endoplasmic reticulum. Cannabis caused DNA damage of polymorphonuclear leucocytes (PNL) evaluated by alkaline single cell gel electrophoresis (comet assay). The above effects of cannabis were dose-dependent [35]. Glial fibrillary acidic protein (GFAP) is a marker of reactive gliosis, a process, whereby, astrocytes became activated in response to brain injury [36]. Studies showed increased GFAP staining in the hippocampus and parietal cortex in rats treated with THC during adolescence [37]. Moreover, rats given cannabis extracts also showed significant increments in brain and serum levels of GFAP [38] (Table 1).

Table 1. In vitro and in vivo cellular toxic effects of THC or cannabis extracts

Experimental model	Toxic effect	Suggested mechanisms	Study
Endothelial cells exposed to marijuana smoke containing THC	↑ Necrotic cell death	↓ Cellular GSH ↑ ROS	[24]
A549 cells treated with THC (2.5 or 10 μg/ml)	↑ Cell death	↓ ATP ↓ Mitochondrial membrane potential	[25]
Rat hippocampal slices exposed to THC (0.5 μM)	↓ Size of neuronal cell bodies & Genomic DNA breakage		[26]
Murine ConA-activated splenocytes and LPS-activated peritoneal macrophages exposed to THC (10 μg/ml)	↑ DNA fragmentation Apoptosis	Caspase-1 mediated ↓ Bcl-2 mRNA and protein	[29]
Primary rat cortical neurons treated with THC	↑ Neuronal apoptosis	↑ Caspase-3 ↑ Bax expression ↑ c-jun N terminal kinase ↑ Cytochrome c	[30][31]
Primary rat cortical neurons treated with THC (5 μM)	↑ Apoptotic cell death	↑ Lysosomal membrane permeability ↑ Caspase-3 Release of the lysosomal cathepsin-D into the cytosol	[32]
Rats treated with THC (10 or 20 mg/kg orally) for 90 days	↓ Dendritic length of CA3 pyramidal neurons.		[33]
Rats treated with THC for 8 months	↓ Neuronal density in hippocampus	↑ Glial cell reactivity	[34]
Rats treated with cannabis extracts (10-20 mg/kg) THC for 4 weeks	↑ Dark neurons with small or undefined nuclei Cellular infiltration Gliosis ↑ DNA damage of PNL (comet assay).	↑ Caspase 3	[35]
Rats treated with THC during adolescence	↑ Glial fibrillary acidic protein (GFAP) staining in the hippocampus and parietal cortex		[37]
Rats treated with cannabis extracts (10-20 mg/kg) THC for 6 weeks	↑ Brain & serum GFAP		[38]

3 Cannabis in Animal Models of Brain Neurotoxicity

In an in vivo model of excitotoxicity induced by intracerebral injection of Na+/K+-ATPase inhibitor ouabain in neonatal rats, co-injection of THC (1 mg/kg, i.p.) exerted neuroprotective effects. Diffusion-weighted magnetic resonance imaging showed that THC reduced the volume of cytotoxic edema (neuronal swelling) 15 min after ouabain injection and the volume of infarcted tissue after 7 days. THC reduced neuronal damage via a CBR1-dependent mechanism [39].

Hayakawa et al. [40] suggested that acute but not chronic administration of THC protects against ischaemic brain injury. In this study, THC (3 and 10 mg/kg) and also cannabidiol significantly reduced the infarct volume induced by middle cerebral artery occlusion for 4h in mice. THC (3 and 10 mg/kg) was injected i.p. immediately before and 3 h after cerebral ischaemia. The neuroprotective effect of acutely administered THC was inhibited by CBR1 antagonist and is likely to involve an increase in

cerebral blood flow and hypothermic effect. In contrast to acute treatment, repeated i.p. injection of THC (10 mg/kg) for 14-day prior to cerebral ischaemia in mice significantly increased infarct volume.

In another study, transient global cerebral ischaemia was induced in rats by bilateral common carotid artery (CCA) occlusion for 45 minutes followed by 4 h of reperfusion. Cannabis extract was i.p. administered in a single dose of 20 mg/kg THC either before CCA, at time of reperfusion, after reperfusion or daily for 2 days before surgery. Cannabis was found to significantly increase GSH and to alleviate the increase in nitric oxide levels in the ischaemic brain tissue. Cannabis given before or at time of CCA occlusion also significantly reduced brain tumour necrosis factor-α (TNF-α). Cannabis given as a single dose 1h before cerebral ischaemia or as two days pretreatment conferred histologic protection against the ischaemic/reperfusion neuronal injury. In contrast, cannabis given at time of CCA occlusion exacerbated brain damage with widespread severe spongiform changes and neuronal loss [41].

Interestingly, cannabis resin extract was found to protect against neurotoxicity caused in the rat by the organophosphorus compound malathion. The extract was given at doses of 10 or 20 mg/kg (expressed as THC) 30 min prior to i.p. injection of malathion. Cannabis did not alter MDA level but prevented the depletion of in GSH and the decrease in paraoxonase-1 activity in the brain of malathion-treated animals. Spongiform changes, neuronal damage in cerebral cortex and the degeneration of Purkinje cells in cerebellum were prevented by cannabis 20 mg/kg [42]. Cannabis was shown to modulate the activities of cholinesterases in brain and serum of rats [43,44]. THC has also been reported to competitively inhibit acetylcolinesterase (AChE) by binding to the allosteric peripheral anionic binding site of the enzyme [45]. Several terpenoids in the plant Cannabis sativa e.g., pulegone, limonene, and limonene oxide were also shown to inhibit AChE in vitro [46]. THC and cannabis other constituents thus might act to prevent the irreversible binding of the organophosphate metabolites onto the AChE and thus prevent the malathion induced neurotoxicity [42].

Other studies, however, failed to demonstrate a neuroprotective effect for cannabis extracts on brain histology despite evidence of reduced brain oxidative stress. One study investigated the effect of cannabis on $AlCl_3$ neurotoxicity was at neurobehavioral, biochemical and histopathological levels. $AlCl_3$ induced brain oxidative stress, a cognitive deficit detected by the water maze test and brain damage in the form of shrunken neurons with pyknotic nuclei and eosinophilic cytoplasm. Cannabis sativa extract (10 and 20 mg/kg THC) was given daily in combination with $AlCl_3$ for 6 weeks. Cannabis significantly alleviated the increase in MDA, nitric oxide and the GSH depletion in brain of $AlCl_3$-treated rats. Cannabis, however, failed to alter the cognitive deficit or the damage in cerebral cortex and hippocampus induced by $AlCl_3$ [47]. In the epilepsy model induced in rats by the GABA (A) receptor antagonist pentylenetetrazole (PTZ), cannabis treatment (20 mg/kg THC) caused significant elevation of seizure scores. In PTZ treated rats, cannabis resulted in significant increase in brain MDA. Histopathological changes induced by PTZ such as degenerated and necrotic neurons, inflammatory cells, and gliosis in cerebral cortex and cerebellar Purkinje cells degeneration were not improved by cannabis treatment [48]. In brain of thioacetamide-treated rats, the increments in MDA and nitric oxide were significantly decreased by cannabis (10 and 20 mg/kg THC). Brain sections from only thioacetamide-treated rats showed some neurons with dark small nuclei. Cannabis enhanced the damaging effect of thioacetamide with neurons showing ballooning and degeneration and increased number of neurons with dark nuclei [49]. Rotenone, a naturally occurring pesticide of plant origin [50] is used in rodents to model human Parkinson's disease [51,52]. In mice, daily i.p. injection of rotenone, the inflammogen LPS or their combination for 2 weeks increased oxidative/nitrosative stress in brain and induced nigrostriatal neuronal damage. Cannabis (20 mg/kg THC) s.c., co-injected with the toxicants, reduced brain oxidative stress but did not reduce neuronal damage [53]. In another study, cannabis resin extract was s.c. given for 2 days prior to and at the time of i.p. LPS endotoxin injection administration. Cannabis 20 mg/kg (expressed as THC) lessened the increase in MDA, nitric oxide and restored GSH levels in the brain of LPS-treated mice. Cannabis, however, increased histologic brain damage with cellular atrophy, shrinkage, necrosis, pyknosis, and deeply stained and dark nuclei being observed in sections from the cerebral cortex. Caspase-3 immunostaining was markedly increased in degenerating neurons of the cerebral cortex by LPS/cannabis compared with only LPS treatment [54].

4 Cannabis and Brain Oxidative Stress

Oxidative stress is the term applied to the situation in which the balance between reactive oxygen species (ROS) and antioxidant mechanisms in the cell is tilted in favor of the oxidant side. This occurs because of either too much ROS or insufficient antioxidants. The result is oxidative damage to cell biomolecules eg., membrane lipids, enzymes, and DNA, cell perturbation and even death [55]. Reactive species are produced within the cell during normal metabolism eg., the mitochondrial respiratory chain where the mitochondrial complexes I and III leak electrons to O_2 forming superoxide anion radical ($O_2^{\cdot-}$). Superoxide can then result in the formation of several intermediates such as hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) or react with nitric oxide to generate the highly reactive peroxynitrite ($ONOO^-$) [56.57]. The mitochondria is the major source for ROS in the cell but is also subject to attack by ROS that may cause mitochondrial dysfunction and initiation of cell death cascade [58] and mitochondrial dysfunction has been linked to aging and age-related neurodegenerative diseases [59,60].

4.1 Human Studies

There are few studies that have measured oxidative stress biomarkers in cannabis smokers. The effects of cannabis were studied by Toson [61] in a group of long-term marijuana smokers with smoking duration of 5-10 years. Compared to healthy controls, cannabis smokers had significant increase in blood malondialdehyde (MDA), a marker of lipid peroxidation. Serum levels of nitric oxide were markedly raised as well. Meanwhile, reduced glutathione (GSH) in blood, and the total antioxidants capacity (TAC) in serum were significantly reduced in cannabis users. Moreover, serum C-reactive protein showed 40% increase in users of cannabis. Other researchers found significant increase in serum MDA and urinary 8-hydroxydeoxyguanosine (a biomarker of oxidative DNA damage) and decreased serum TAC in tobacco and tobacco/marijuana smokers compared to healthy controls [62]. Bayazit et al. [63] measured total antioxidant status, total oxidant status and proinflammatory cytokines levels in serum in patients with cannabis use disorder. Patients aged between 18 and 35 years of age and were using cannabis at time of study. Compared with their healthy controls, cannabis-dependent subjects exhibited significant increase in total oxidant status, and oxidative stress index. Moreover, there were significant increments in the interleukins 1β, 6, 8, and tumor necrosis factor-α in subjects with cannabis use disorder. Another study in patients with cannabis dependence reported a significant increase in lipid hydroperoxide, and decreased free thiol levels but increased brain-derived neurotrophic factor, and ceruloplasmin in serum [64].

In contrast to the above studies, Bloomer et al. [65] reported no significant effect for smoking marijuana on oxidative stress. In their study, young (23-24 years) frequent marijuana users and non-smokers participated in regular exercise and then tested for biomarkers of oxidative stress and cardio-metabolic health. Participants smoke marijuana on average of 4.5 ± 2.3 sessions per week for at least three months before the study. The study found no significant differences in MDA or advanced oxidation protein produces in plasma of marijuana users compared with non-users which was explained by the beneficial effect of regular exercise on oxidative stress (Table 2).

Table 2. Human studies indicating increased oxidative stress or inflammatory markers in cannabis users

Oxidative stress biomarkers	**Study**
↑ MDA in blood ↑ Nitric oxide in serum ↓ GSH in blood ↓ TAC in serum	[61]
↑ MDA in serum ↑ Urinary 8-hydroxydeoxyguanosine ↓ TAC in serum	[62]
↑ Total oxidant status ↑ Oxidative stress index.	[63]
↑ Lipid hydroperoxide in serum ↓ Free thiols in serum	[64]

Inflammatory markers	
↑ C-reactive protein in serum	[62]
↑ IL-1β in serum ↑ IL-6 in serum ↑ IL-8 in serum ↑ TNF-α in serum ↑ Ceruloplasmin in serum	[63]

Abbreviations: MDA (malondialdehyde); GSH (reduced glutathione); TAC (total antioxidant capacity); IL-1β (interleukin-1beta); IL-6 (interleukin-6); IL-8 (interleukin-8); TNF-α (tumour necrosis factor- α).

4.2 In Vitro Studies

Sarafian et al. [24] exposed endothelial cell line to smoke produced from marijuana cigarettes containing either 1.77 or 3.95% THC. Marijuana smoke from 3.95% THC cigarettes resulted in a rapid and sustained decrease in cellular GSH level by 83% after 10 min. Marijuana smoke (though not pure synthetic THC) increased ROS by 80%. Several research groups have shown that THC causes mitochondrial dysfunction and increase ROS. In isolated mitochondria extracted from rat brain, exposure to THC inhibited complexes I, II, and III of the mitochondrial respiratory chain and decreased mitochondrial coupling. THC enhanced H_2O_2 production and mitochondrial free radical leak [28]. THC was also shown to deplete ATP, impair mitochondrial respiratory function, decrease mitochondrial membrane potential, increase cellular ROS and markers of lipid damage in human trophoblast cell lines. THC also increased mitochondrial fission and the expression of cellular stress markers, HSP70 and HSP60 [66].

In contrast, in vitro experiments in a Fenton reaction system, cannabidiol and THC were able to prevent Tert-butyl hydroperoxide-induced oxidation of dihydrorhodamine, an oxidation sensitive fluorescent dye. THC and cannabidiol were shown to equally protect rat cortical neurons in culture against glutamate neurotoxicity (decrease LDH release) [67,68]. *N*-methyl-D-aspartate (NMDA)-induced cell death in AF5 rat mesencephalic cell line was also prevented by THC (by CBR1 independent mechanism) (3 μM) [69]. THC exerted antioxidant and anti-apoptotic effects and significantly reduced dopaminergic cell death in culture induced by 1-methyl-4-phenylpyridinium (MPP+), lactacystin and paraquat. These effects of THC were blocked by peroxisome proliferator-activated receptor-gamma (PPARγ) antagonist [70]. Using differentiated human neuronal SY-SH5Y cells exposed to H_2O_2 or amyloid-β1–42 (Aβ1–42) in the presence of Cu (II), Rajia et al. [71] reported reduced ROS formation by cannabis extracts with high (72%) THC content.

4.3 Animal Experiments

The effect of cannabis on oxidative stress is complex with both antioxidant and prooxidant effects being reported. Vella et al. [72] investigated the effect of 8 weeks administration of a small dose THC (0.15 mg/kg, daily, i.p.) in diabetic rats. There was marked decrease in serum MDA and NO in control rats. The increase in serum MDA in diabetic rats was significantly decreased by treatment with THC which also decreased IL-1β and increased IL-6 in serum. Coskun and Bolkent [73] found that rats treated with THC (3 mg/kg, ip.) daily for one week exhibited significant increase in MDA in the plasma. The erythrocyte GSH levels, plasma catalase or superoxide dismutase, however, were not changed. On the other hand, treatment of diabetic rats with THC resulted in significant decrease in plasma MDA. Moreover, erythrocyte GSH levels and plasma superoxide dismutase levels were significantly increased in the diabetic group treated with THC compared with the control diabetes group. The study showed that THC acted as antioxidant in diabetic but not in normal rats. Ebuchi and Solanke [74] treated rats with 25 mg/kg marijuana extract for two weeks. The authors reported significant increase in MDA and a decrease in GSH levels in rat brain and liver. The activities of the antioxidant enzymes superoxide dismutase and catalase decreased as well after treatment with the marijuana extract. Khadrawy et al. [75] investigated the effects of cannabis extract rich in THC in the model of reserpine induced depression in rats. *Cannabis sativa* extract (10 mg/kg THC, s.c.) given after 15 days of initiating reserpine and continued together with reserpine for another 15 days found to exacerbate the lipid peroxidation (MDA) in the cortex and hippocampus.

In the study of Kopjar et al. [76] rats were treated with a single THC dose of 7 mg/kg, orally. The authors reported significantly increased

thiobarbituric reactive substances (TBARS) in brain after 1 day of treatment with THC together with significantly elevated brain GSH Level by 37.5%. Serum TBARS decreased by 29.4%. Meanwhile, there was a significantly decreased superoxide dismutase activity in brain of THC-treated rats by 69.8% compared to controls. Kubiliene et al. [77] investigated the effect of marijuana extract on oxidative stress induced in mice brain by AlCl3. The extract was given intragastrically at the dose of 1.6 mg/g for 21 days. Administration of only marijuana extract had no significant effect on levels of reduced glutathione in blood or MDA in brain and liver but significantly increased catalase activity. In ACl_3-treated mice, however, cannabis alleviated the increase in MDA and the decline in catalase activity in brain and liver.

Other studies carried out in rodents using only marijuana or cannabis resin extracts showed a moderate though a significant decrease in brain MDA after treatment with cannabis at the dose of 20 mg/kg THC for 4 weeks [53,78]. Rats given subcutaneous (s.c.) injections of cannabis resin extract for 6 weeks showed significant increments in serum MDA. This was especially so for the lower doses of 5 or 10 mg/kg THC compared with the higher dose of 20 mg/kg [79]. Reduced glutathione was significantly increased in brain and serum by treatment with 20 mg/kg marijuana or cannabis resin extracts [49,54,78,80]. Moreover, brain superoxide dismutase activity and ascorbate level were shown to increase following treatment with cannabis extracts [81].

Nitric oxide is a free radical that when produced in excess can be neurotoxic via mechanisms such as inhibition of cytochrome, excitotoxicity, energy failure, and apoptosis [82]. These effects are mediated through interactions with $O_2^{\cdot-}$ to form peroxynitrite or with O_2 to form nitrogen oxides [83]. Studies showed significant decrease in brain nitric oxide in mice treated with only marijuana extract at 10, 15 or 20 mg/kg (expressed as THC) for 18-30 days [53,78] and in rats treated with cannabis resin extracts at the dose of 20 mg/kg THC for 6 weeks [79]. Moreover, in a study by Vella et al. [72] treatment with THC (15 mg/kg, i.p. for two months) caused a 24.6% decrease in serum nitric oxide. Inhibition of nitric oxide release might therefore account at least in part the ability of cannabinoids to protect neurons from excitotoxic injury [84].

5 Cannabis and Brain Energetic Metabolism

The mechanisms that underlie the detrimental effects of herbal cannabis on brain structure and function are not fully understood. Costa and Colleoni [85] suggested that inhibition of brain energetic metabolism and a low ATP production could be a mechanism by which long-term cannabis causes neuronal injury. The authors found that single i.p. administration of THC (10 mg/kg) in rats increased brain mitochondria oxidative phosphorylation via the cannabinoid CB1 receptor. Lipoperoxide levels in cerebral cortex were also increased possibly due to the increase in brain mitochondria oxygen uptake. In contrast, repeated administration of THC (10 mg/kg, twice daily for 4.5 days, i.p.) decreased the brain mitochondria oxygen consumption and uncoupled oxidative phosphorylation. In pig brain mitochondria in vitro, THC (also cannabidiol and anandamide) was shown to inhibit mitochondrial respiration [86].

The transcription factor, nuclear respiratory factor-2 (NRF-2), also known as GA-binding protein, mediates the expression of a number of nuclear-encoded mitochondrial proteins required for mitochondrial respiratory function and oxidative phosphorylation. NRF-2 is important for the control of mitochondrial biogensis and functions. Loss of NRF-2 results in reduced mitochondrial mass, oxygen consumption and consequent decrease in ATP production and mitochondrial protein synthesis [87,88]. It has been shown in rats that treatment with cannabis extract for 6 weeks was associated with significant decrease in serum NRF-2, thereby, suggesting that cannabis could affect mitochondrial biogenesis and activity [79].

Studies in mice during infancy have also shown a detrimental and long-lasting effect for THC on brain bioenergitics. In this context, the repeated exposure of preadolescent healthy mice to THC (0.5 mg/kg) i.p. daily for 11 consecutive days disrupted the expression of mitochondrial proteins (complexes I-IV), and induced loss of membrane integrity occluding mitochondrial respiration [89]. In another study, the single administration of THC in 10-day-old mice (10 and 50 mg/kg) was found to affect the transcript levels of genes involved in neurotrophic and oxidative stress signaling 24 h after exposure ie., decreased neurotrophic receptor Trkb transcript levels and increased Nrf2/Keap1 ratio in parietal cortex and hippocampus. The pro-apoptotic marker BAX was also increased in the frontal cortex [90]. It

was also found that administration of THC (1 or 5 mg/kg) daily to preadolescent mice caused disrupted expression of mitochondrial proteins (complexes I-IV), and induced loss of membrane integrity occluding mitochondrial respiration in hippocampal neurons. These effects of THC lasted more than 4 months [91].

Using positron emission tomography, Volko et al. [92] reported decreased cerebellar metabolism chronic marijuana abusers compared with normal subjects, which could explain the motor deficits in marijuana users. Rats treated with cannabis showed a significant decrease in brain glucose which might impair brain energetic and account for the effect of cannabis on memory functioning [78]. Other researchers found that small doses (≤ 0.01 mg/kg) and low blood levels of THC (< 1 ng/ml) were associated with increased glucose uptake especially in the hypothalamus. In contrast, higher doses (≥ 0.05 mg/kg) and blood levels (> 10 ng/ml) resulted in decreased glucose uptake, especially in the cerebellar cortex [93]. Cannabis (54 mg) given to healthy cannabis users either orally, through smoking (6.9%THC cigarette) or via inhalation of heated vaporized cannabis (Volcano®) caused significant increases in plasma ammonia concentrations which positively correlated with THC concentrations in blood. Experiments in mice indicated that THC (3 and 10 mg/kg, i.p.) significantly reduced striatal glutamine synthetase activity, and increased striatal ammonia concentration followed by significant increase in plasma ammonia. The THC-induced increase in brain ammonia might be neurotoxic [94].

6 Cannabis and Brain Inflammation

In rats, THC (0.5, 1.0 and 2.0 mg/kg, i.p.) was found to increase brain concentrations of prostaglandins E2 and F2 alpha [95] while repeated administration of THC (10 mg/kg) in mice increased brain cyclooxygenase-2 expression [96], suggesting that cannabis may cause brain inflammation. In female rats, chronic administration of increasing doses of THC during adolescence induces a persistent neuroinflammatory state in the adult prefrontal cortex. There were increased expression of the pro-inflammatory cytokine TNF-α, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and decreased anti-inflammatory cytokine interleukin-10. Inhibition of microglia activation inhibited during THC treatment prevented the neuroinflammatory state and attenuated short-term memory impairments in adulthood [97].

7 Conclusion

There is evidence from both human studies and animal research which strongly supports a detrimental effect for THC-rich cannabis on neuronal integrity. As for the role of oxidative stress, although cannabis users showed elevated blood levels of oxidative stress, in vitro and animal experiments were less conclusive. Clearly, there is a need for further research in this context.

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