ROS

REVIEW ARTICLES

Oxidative Stress and the Combined Use of Tetrahydrocannabinol and Alcohol: Is There a Need for Further Research?

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ABSTRACT | *Cannabis sativa* is a plant that produces, among other cannabinoids, the psychoactive Δ-9-tetrahydrocannabinol (THC), whose adverse health effects have been recently reviewed. Repeated doses of THC lead to the development of tolerance to its own effects and the effects of other cannabinoids, and affect the same reward systems as alcohol, cocaine, and opioids. Enhanced brain oxidative stress usually correlates with cognitive impairment and a higher risk of development of neurodegenerative diseases, which has been repeatedly reported for many conditions. THC-induced cognitive effects are aggravated by the combined use of alcohol. Some research suggests similar mechanistic aspects of THC and alcohol on brain mitochondrial oxidative damage. Taking THC and ethanol together represents a greater risk for cognitive and attention impairment than taking either drug separately. While THC and ethanol exposure elevates reactive oxygen species in many tissues including the brain, further research is needed to establish a deleterious role of oxidative stress in the nervous system subjected to both compounds.

KEYWORDS | Alcohol; Cannabis; Oxidative stress; Cognitive impairment

ABBREVIATIONS | ADH, alcohol dehydrogenase; CAT, catalase; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CNS, central nervous system; CYP2E1, cytochrome P450 2E1; ECS, endocannabinoid system; EtOH, ethanol; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; THC, Δ-9-tetrahydrocannabinol

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1. INTRODUCTION

It is well known that among *Cannabis sativa* preparations, marijuana (the dried flowers and subtending leaves and stems of the female cannabis plant) and hashish (a concentrated resin or ball produced from the detached trichomes and fine material that falls off cannabis flowers/leaves) are the most popular and commonly used illicit drugs worldwide. The last European Drug Report, published in 2016, confirmed that cannabis use is about 5 times higher than other substances and also the number of consumers who get treated for cannabis problems has increased considerably in recent years [1]. It is estimated that almost 1/4 of the adult population of the EU, over 88 million people, have tried illegal drugs at some point in their lives and the most consumed is cannabis with 83.9 million users (95.34% of total consumers of illegal drugs). The use and possession of cannabis for personal consumption represents over 74% of violations of drug laws reported in the EU [1].

A very comprehensive review has been recently published on the adverse health effects of marijuana [2]. These authors report on how legal drugs such as alcohol and tobacco account for the greatest burden of diseases associated with drugs. However, the potential future increase in the use of marijuana raises the question, not only of its adverse health effects as they are elegantly reviewed [2], but also of the adverse health effects of the combined use of marijuana and the other most consumed legal drug—alcohol (chemically known as ethyl alcohol or ethanol).

The cannabis plant produces cannabinoids which are a group of C_{21} terpenophenolic compounds, and the primary psychoactive constituent is Δ-9-tetrahydrocannabinol (THC) [3]. THC is one of the over 480 constituents of marijuana, and it is the main psychoactive component of marijuana. In fact, marijuana potency is generally measured by the percentage of THC content in the product—hashish oil has 20% of THC; hashish has 5–15% of THC, and marijuana has 1–5% THC. Other marijuana components include more than 60 other cannabinoids as well as many other different chemical compounds. One of those cannabinoids is cannabidiol. It offsets some of the psychoactive THC effects, due to its antipsychotic and antianxiety effects [4]. THC acts as a partial agonist of cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2) of the endocannabinoid system (ECS) [5]. Thalamic nuclei, known to be involved in behavioral and cognitive function (anterior, mediodorsal, midline, and intralaminar), have a higher receptor density, as do other limbic regions, including the hippocampus, amygdala complex, and entorhinal cortex [6]. Important pharmacological activities of cannabinoids seem to be mediated by the CB_1 receptor, since CB_1 receptor is the most abundant in the central nervous system (CNS) and, to a lesser extent, in peripheral tissues [7]. THC major metabolic pathways are represented in **Figure 1**.

Repeated doses of THC lead to the development of tolerance to its effects, and apparently cannabinoids may affect the same reward systems as alcohol, cocaine, and opioids [8]. Although cannabidiol has been claimed to have antioxidant and even neuroprotective properties [9, 10], a recent publication has clearly demonstrated the induction of brain mitochondrial dysfunction and oxidative stress by THC treatment [11].

The hazardous consumption of alcohol is a contributing factor to death all over the world. Worldwide, 3.3 million deaths every year result from harmful use of alcohol, which represent 5.9% of all deaths. Furthermore, the harmful use of alcohol is a causal factor in more than 200 disease and injury conditions, according to the World Health Organization [12]. Hence, alcohol abuse-related disorders are correlated with a wide spectrum of, psychological, behavioral, social, and other medical problems [13]. Over time, alcohol has become the most socially accepted addictive drug worldwide [14].

2. EVIDENCE OF OXIDATIVE STRESS GENERATION

2.1. THC

Toxic effects of multiple drugs and also of drugs of abuse are often associated with oxidative stress, among other mechanisms. In this regard, recent and not yet extensive research on different cannabinoid compounds is available that leads to two main opinions. On the one hand, many reports show positive effects of cannabidiol. However, THC accounts for a majority of literature data suggesting its deleterious effects in nervous tissue; those related to oxidative stress are herein reviewed, though not much information is available on the oxidative effects of marijuana or THC.

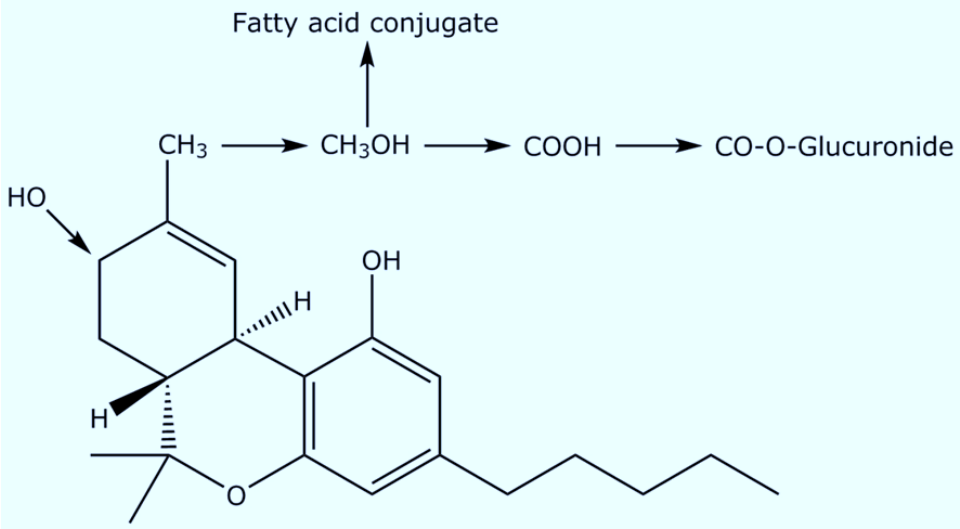

FIGURE 1. THC chemical structure and major metabolic modifications. THC is first metabolized by enzymatic hydroxylation to form 11-hydroxy-THC and then by enzymatic oxidation to yield THC-11-oic acid, which forms a conjugate with glucuronide.

In zebra mussel (*Dreissena polymorpha*) 0.5 μg/L Δ-9-THC exposure caused significant alterations in its oxidative status, accompanied by significant increases of lipid peroxidation, protein carbonylation, and DNA damage [15]. THC induces an increased dopamine and noradrenaline release in mice striatal, nucleus accumbens, and prefrontal areas of the brain [16, 17], whereas in healthy volunteers THC induces dopamine release in striatum [18]. Considering the oxidative stress implications of dopamine metabolism [19, 20], the increased dopamine release may contribute to THC-related oxidative stress enhancement. These effects of brain monoamines could account for the cognitive impairment in cannabis users. Alterations in the endocannabinoid system, which appears to influence development processes by modulating neurotransmitters produced by marijuana use, can lead to changes in the neural circuits (neurochemical and structural changes). This affects synaptogenesis, pruning, and myelination [21, 22] resulting in cognitive and emotional changes. In a somewhat more complicated animal model—the simian immunodeficiency virus (SIV)-infected macaque, it has been very recently reported and speculated that the treatment of these infected animals with THC downregulates the number of NADPH oxidase 4 (NOX4)-positive intestinal cells, mediated by microRNA miR-99b. It is possible that this could be the mechanism of the intestinal epithelium protection from oxidative stress-induced damage observed in the non-treated infected animals [23]. However, in cell culture models, different authors confirm THC-mediated negative effects. For example, it has been demonstrated that the cytotoxicity of THC to J774-1 cells is exerted through the CB_2 receptor, followed by the activation of p38 mitogen-activated protein kinase (MAPK) [24]. It has also been shown that CB_1 receptor activation with the endocannabinoid anandamide or synthetic agonist HU210, promotes cell death in human primary coronary artery endothelial cells by the amplification of the reactive oxygen species (ROS)–MAPK activation–cell death pathway, in an in vitro inflammatory model [25]. Furthermore, cannabidiol and THC (to a less extent) induce a cellular stress response mediated by nuclear factor kappa B (NF-κB) and nuclear factor (erythroid-derived 2)-like 2 factor (Nrf2), both related to oxidative status, and that this response underlies their high immunosuppressant activities in microglial cells [26].

Oxidative stress markers in the brain have been studied since oxidative stress has been implicated in memory impairment [27]. Enhanced brain oxidative stress usually correlates with cognitive impairment

[28] and with a higher risk of development of neurodegenerative diseases [29]. High concentrations of THC can increase lysosomal permeability through CB_1 receptor binding, which may be responsible for its neurotoxicity [30]. An increase in brain mitochondrial oxidative phosphorylation was shown ex vivo in THC-treated rats, and it was antagonized by SR141716A, a CB_1 receptor blocker [31]. In this regard, and as mentioned above, this THC-induced brain mitochondrial dysfunction associated with increased oxidative stress has been recently confirmed and directly related with an increased risk for cannabis-related stroke [11].

2.2. Ethanol

The acute and chronic consumption of ethanol (EtOH) leads to higher production of ROS and enhances the peroxidation of lipids, proteins, and DNA in a variety of systems, cells, and different species, including humans [32]. Although the main EtOH-induced alterations take place in the liver, there are also strong indications of the existence of an extrahepatic oxidative EtOH metabolism in organs such as the brain, heart, kidneys, and stomach. EtOH exerts its harmful effects metabolically via oxidative (acetaldehyde) and non-oxidative (ethyl esters of fatty acids) pathways [33], resulting in free radical production and lipid peroxidation [33–37].

The principal EtOH-metabolizing enzymes are alcohol dehydrogenase (ADH), catalase (CAT), and cytochrome P450 2E1 (CYP2E1) [33]. ADH, a zinc-containing enzyme localized in the cytosolic compartment of the cell, seems to oxidize EtOH from different sources—endogenous EtOH produced by microorganisms in the intestine; exogenous EtOH and other alcohols consumed in the diet; substrates involved in steroid and bile acid metabolism; and other primary or secondary alcohols [33]. Metabolism of EtOH by ADH can increase oxidative stress. High ADH activity can be found in the liver, brain, gastrointestinal tract, kidneys, nasal mucosa, testes, uterus, and ocular tissues [38–41].

CAT can also oxidize EtOH. This pathway is limited due to the low rates of H_2O_2 generation under physiological cellular conditions, thereby having an insignificant role in EtOH oxidation in the liver though a number of the CNS effects of EtOH are mediated by acetaldehyde [42]. It has been shown that CAT is also present inside the brain's peroxisomes. In this context, CAT inhibitors were reported to reduce oxidation of EtOH to acetaldehyde in the brain [43].

The P450s are heme proteins, consisting of amino acids that fold in a way that they surround a prosthetic group containing iron [44]. There are many contributions demonstrating EtOH induction of oxidative stress in nerve cells. These include intracellular redox state changes, production of acetaldehyde, damage to mitochondria, direct effect on cell membrane caused by hydrophobic EtOH interactions, altered cytokine production, effects on antioxidant enzymes and chemicals, impairment of neurogenesis, and other pathways [33, 45–49]. Nonetheless, the main pathway appears to be the induction of CYP2E1 by EtOH, being an effective generator of ROS [33]. Despite the fact that EtOH is catabolized mostly in the liver, the presence of CYP2E1 in other tissues reinforces the possibility of an extrahepatic EtOH tissue metabolism. Although the contribution of CYP2E1 to total alcohol metabolism in basal conditions is small, if induced it plays a very significant role in the generation of oxidative stress [42, 50, 51].

3. ADVERSE HEALTH EFFECTS OF COMBINED USE

Marijuana use has been associated with substantial adverse effects, some of which have been determined with high-medium level of confidence. These include addiction (to marijuana and other substances), abnormal brain development, progression to use of other drugs, schizophrenia, depression and anxiety, diminished lifetime achievement, motor vehicle accidents, and symptoms of chronic bronchitis [2]. Different mechanisms have been suggested for THC toxicity, including an increase in oxidative stress, but the association between the oxidative status in the brain and THC-induced behavioral changes is poorly understood.

Simons and collaborators showed that consumers of alcohol and marijuana (together) consumed more alcohol compared to people who only consumed alcohol. Thus, the consumption of both is associated with a greater amount of alcohol intake. Also, those who consumed alcohol and marijuana together had more problems related to alcohol, compared to people who only consumed alcohol [52]. In another study, cannabis use was associated with a higher risk

of developing alcohol-related disorders [53]. Furthermore, when cannabis and alcohol are detected together, there is a greater risk to road safety than when either drug is used alone [54, 55].

According to the reports of Susan Tapert's group, adolescents who consume sporadically large quantities of alcohol and/or marijuana have a poorer integrity of white matter at 20 years of age, compared with adolescents with minimal consumption. The continuous decline in cognitive performance may be related to the fact that monthly consumption varies from 17 to 45 alcoholic beverages [56]. These authors concluded that: (1) adolescents with no history of alcohol and/or marijuana have greater integrity of the white matter, compared with drinkers/marijuana smokers; and (2) teens that were classified as "binge drinkers" showed the same neurostructural changes as those who consumed alcohol and/or marijuana in large quantities on a regular basis [57].

Other reports of the above same group also showed changes in the integrity of the macrostructure (e.g., cortical thickness) [58], in the patterns of neural activation [59], and decreases in cognitive status [60, 61]. Furthermore, they also demonstrated that the combined use of marijuana and EtOH increased cortical thickness in all four lobes of the brain, bilaterally. Notably, 18 of 23 regions in which differences were observed were in the frontal and parietal cortex. Positive dose-dependent associations were identified in temporal brain regions, as cumulative marijuana use from ages 16 to 22 was associated with thicker cortices in inferior temporal and entorhinal cortex. Several negative associations were observed with lifetime alcohol use; higher alcohol use was reported to be associated with thinner cortical estimates in all four lobes [56].

A very recent review by Lipina and Hundal [62] has extensively compiled the evidence of the cross talk between the endocannabinoid system (ECS) and ROS in the cellular environment, since both are very prominent signalling systems. It is clear, as these authors suggest that modifications in either of them may contribute to the genesis and/or further development of various diseases. As outlined in the present review, EtOH metabolism in different tissues as an extra (and often times uncontrolled) source of ROS, and THC, as a partial agonist of CB_1 and CB_2 receptors [5], have the ability to dysregulate this fine tuning between ROS and the ECS. This may occur with either compound or both together.

As confirmed herein, not enough information is available on the oxidative effects of combined EtOH and marijuana/THC use. Interestingly, brain mitochondrial damage, leading to oxidative stress, seems to occur both under EtOH [47] and THC treatments [11], but the experimental approach of the combination is still missing. Similarly, the proposed effects of THC consumption on body weight, body mass index and fat distribution (including hepatic fat), are not yet conclusive [63]. However, some authors have found elevated hepatic enzymes and eventually hepatomegaly in marijuana consumers [64], which certainly would deserve further research to test whether any type of deleterious synergistic effect could exist with combined alcohol consumption, mediated by oxidative mechanisms, in view of the well-known hepatotoxicity of ethanol mediated by oxidative stress.

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