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Increased brain and serum nuclear factor kappa-B levels by cannabis and tramadol

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ABSTRACT

Nuclear factor kappa-B (NF-κB) is transcription factor that controls the expression of several genes involved in inflammation and immunity. The aim of the present study was to evaluate the levels of NF-κB in brain and serum of rats treated with *Cannabis sativa* resin, tramadol or their combination. Rats were treated with **c**annabis resin extract (5, 10 or 20 mg/kg) (expressed as Δ^9-tetrahydrocannabinol), tramadol (5, 10 or 20 mg/kg) or tramadol (10 mg/kg) combined with cannabis (5, 10 or 20 mg/kg) subcutaneously (s.c.) once a day, for 6 weeks. Results showed that NF-kB was significantly increased in the brain by 21.8-84.3% after treatment with 5-20 mg/kg cannabis and in serum by 37.7% and 70.8% after treatment with 10 or 20 mg/kg cannabis. NF-κB was also significantly increased in the brain by 25.7-48.2% following treatment with 5-20 mg/kg tramadol and in serum by 34.8% after 20 mg/kg tramadol. After the combined administration of both cannabis and tramadol, NF-κB levels were significantly raised in the brain by 45.5-101.2% and in serum by 40.7-91.8%. The increase in brain tissue and serum levels of NF-kB in rats treated with cannabis and/or tramadol suggest that NF-κB may play a role in the pathogenesis of neuronal injury by these drugs of abuse.

Keywords: Tramadol, cannabis, nuclear factor kappa-B, oxidative stress, neurodegeneration

1. INTRODUCTION

Cannabis from the plant *Cannabis sativa* is the most abused drug world-wide, with about 192 million people reported using it in 2018 (World Drug Report, 2020). Marijuana is the term used to describe the flowering tops and leaves of the plant while hashish refers to the compressed resin (Ashton, 2001). When smoked in cigarettes or water pipe, cannabis produces a sense of mild euphoria, relaxation, enhancement of sensory perception and altered time perception (Huestis, 2002). The chronic use of cannabis, however, is associated with adverse health effects. Cannabis impairs memory processing (Solowij et al., 2002) and heavy users exhibit decline in their cognitive abilities and deterioration in academic achievements (Meier et al., 2015). Frequent and heavy usage of cannabis has also been linked to the development of the more severe mental illness schizophrenia (Di Forti et al., 2014). The recreational as well as the adverse health effects of cannabis were found to be mediated by its major psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) (Mechoulam and Gaoni, 1967) acting mainly on cannabinoid type 1 receptors in brain areas that control memory, cognition, motor activity i.e., cerebral cortex, hippocampus, basal ganglia and cerebellum (Svízenská et al., 2008). Animal studies revealed memory impairing (Niyuhire et al., 2007; Abdel-Salam et al., 2013) and brain neurotoxic effects for cannabis with shrinkage of neuronal cell bodies, gliosis, DNA damage and caspase-3 activation (Abdel-Salam et al., 2014).

In addition to cannabis, several reports indicated the increasing abuse of the analgesic drug tramadol, especially among young adults, which constituted an alarming public health problem world-wide (Grond and Sablotzki, 2004; El-Hadidy and Helaly, 2015; Abdel-Salam et al., 2016; Herrnsdorf et al., 2022). Tramadol is a centrally acting analgesic, a synthetic analogue of codeine with weak agonistic action at the μ-opioid receptor. It also inhibits the reuptake of serotonin and norepinephrine (Grond and Sablotzki, 2004; Miotto et al., 2017). The drug is used in the management of moderate to severe pain eg., post-surgery and cancer pain (Grond and Sablotzki, 2004; Barakat, 2019). In laboratory animals, repeated administration of tramadol resulted in brain neurodegenerative changes eg., shrunken neurons exhibiting dark cytoplasm and pyknotic nuclei, cytoplasmic vacuolations (Abdel-Salam et al., 2019), red neurons (Atici et al., 2004). There was also increased Bax expression in cerebral cortex (apoptosis) (Ghoneim et al., 2014). The mechanisms underlying cannabis and/or tramadol-induced neurodegeneration in the brain are complex and involve reduced glucose availability, impaired mitochondrial functioning and inhibition of brain energetic metabolism (Abdel-Salam, 2022a).

The transcription factor NF-κB is involved in the regulation of expression several genes encoding immune and inflammatory mediators (Haddad, 2002). Under resting conditions, NF-κB is kept in the cytoplasm as an inactive complex by binding to inhibitory proteins IκB. When activated eg., by pro inflammatory cytokines, bacterial lipopolysacchride endotoxin or oxidant free radicals, NF-κB is released from the IκB-NF-κB complex by phosphorylation by IκB-kinase followed by ubiquitination and degradation by proteasome, translocates to the nucleus where it induces the expression of proinflammatory genes encoding for acute phase proteins, cell adhesion molecules, monocyte chemo attractant protein, cytokines, nitric oxide synthase and cycloxygenase-2 (Liu et al., 2017). The aim of the present study was therefore to investigate the effect of cannabis and/or tramadol on NF-κB in the rat.

2. MATERIALS AND METHODS

Animals

Male Sprague-Dawley strain rats, weighing between 140-150g were obtained from the Animal House Colony of the National Research Centre. Animals were group-housed under temperature- and light-controlled conditions and had free access to standard laboratory rodent chow and tap water. The experimental procedures were performed in compliance with the Institutional Ethics Committee and with the guidelines for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and Chemicals

Cannabis sativa resin (hashish) and tramadol were kindly provided by the Laboratory of Forensic Sciences of the Ministry of Justice, Cairo, Egypt. The remaining chemicals and reagents purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A) and were of analytical grade.

Preparation of Cannabis Resin Extract

Cannabis resin extract was prepared from the dried resin of *Cannabis sativa*. The extraction procedure was carried out with chloroform. The method followed that of according to Turner and Mahlberg, (1984) with modification. Briefly, 10 g of the fresh resin were grounded in a mortar and then placed in an oven heated to 100°C for 1h with the aim of decarboxylating cannabinolic acids. Thereafter, the cannabis resin was extracted in chloroform overnight, filtered and the filtrate was evaporated under a gentle stream of nitrogen, stored at 4°C and light protected using an aluminium-covered container. One gram of the dry extract was suspended in 2% ethanol-saline for re-use in the experiments. Δ^9-tetrahydrocannabinol (Δ^9-THC) content was quantified with gas chromatography–mass spectrometry (GC-MS). The resin contained ~ 20% Δ^9-THC and 3% cannabidiol.

Study Design

Rats were randomly divided into 10 equal groups, six rats each:

Group 1 received the vehicle (0.2 ml saline).

Group 2, 3 & 4 were treated with cannabis resin extract at the doses of 5, 10 or 20 mg/kg.

Groups 5, 6, 7 were given tramadol at doses of 5, 10 or 20 mg/kg.

Groups 8, 9, 10 received tramadol at 10 mg/kg in combination with cannabis resin extract at 5, 10 or 20 mg/kg.

Rats were treated with the vehicle, cannabis resin extract, tramadol or cannabis/tramadol combination s.c. once a day for 6 weeks and then euthanized by decapitation under light ether anaesthesia for tissue collection. The brain of each rat was rapidly dissected and snap-frozen in liquid nitrogen, stored at -80°C until further processing.

Quantification of Nuclear Factor Kappa-B

Nuclear factor kappa-B was quantified with an enzyme-linked immune sorbent assay (ELISA) according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA).

Statistical Analysis

Data in the study are expressed as mean ± SE. Data were analyzed by one-way analysis of variance and Duncan's multiple range post-hoc test for comparing group means. SPSS software (SPSS Inc., Chicago, IL, USA) was used. Effects with a probability *p* < 0.05 were considered to be statistically significant.

3. RESULTS

Effect of Cannabis, Tramadol or Cannabis/Tramadol on Brain Nuclear Factor Kappa-B

Compared with the saline-treated group, the brain levels of NF-κB showed significant increase by 21.8%, 68.7% and 84.3% after repeated treatment with 5, 10 and 20 mg/kg cannabis, respectively (89.2 ± 3.9, 123.5 ± 4.7 and 134.9 ± 3.8 vs. 73.2 ± 1.18 U/l). Brain NF-κB also increased by 25.7%, 34.6% and 48.2% by 5, 10 or 20 mg/kg tramadol (92.0 ± 2.1, 98.5 ± 2.2 and 108.5 ± 3.7 U/l vs. 73.2 ± 1.18 U/l). NF-κB levels increased 45.5%, 86.2%, 101.2% of the control by combined treatment with 5, 10 or 20 mg/kg cannabis and 10 mg/kg tramadol (106.5 ± 4.2, 136.3 ± 2.7, 147.3 ± 3.5 vs. 73.2 ± 1.18 U/l).

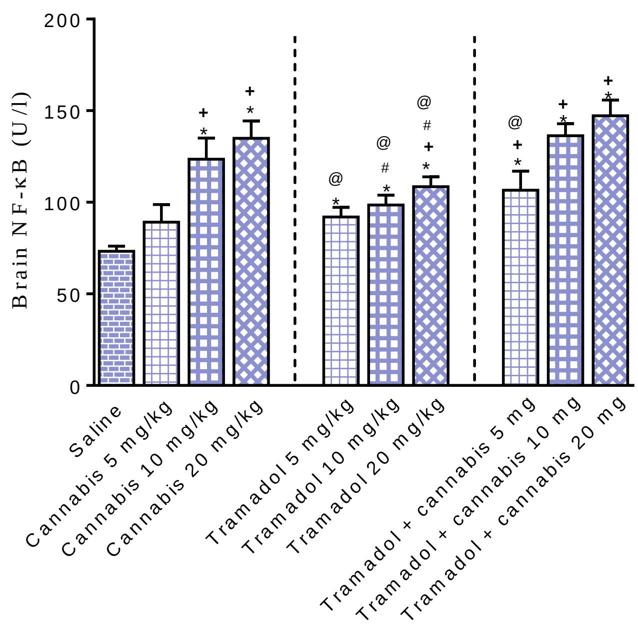

Figure 1 Nuclear factor-kappa B (NF-κB) in the brain of rats treated with different doses of cannabis, tramadol or cannabis/tramadol for 6 weeks. Data are mean ± SEM. *p < 0.05 vs. saline and between other groups as shown on the figure. +p < 0.05 vs. only cannabis 5 mg/kg, @p < 0.05 vs. cannabis 20 mg/kg, #p < 0.05 vs. tramadol 5 mg/kg

Effect of Cannabis, Tramadol or Cannabis/Tramadol on Serum Nuclear Factor Kappa-B

Serum levels of NF-κB were also significantly increased by 37.7% and 70.8% in rats treated with 10 or 20 mg/kg cannabis compared to the saline group (216.5 ± 3.5, 276.7 ± 6.3 and 343.1 ± 7.3 *vs.* 200.9 ± 5.5 U/l). NF-κB in the serum of rats receiving tramadol at the dose of 20 mg/kg increased by 34.8% compared to the control (217.9 ± 6.1 and 270.8 ± 7.6 *vs.* 200.9 ± 5.5 U/l). Following the concomitant treatment with cannabis and tramadol, serum NF-κB increased by 40.7%, 86.8%, and 91.8% in comparison with the control (282.6 ± 10.0, 375.2 ± 10.9 and 385.4 ± 7.8 *vs.* 200.9 ± 5.5 U/l).

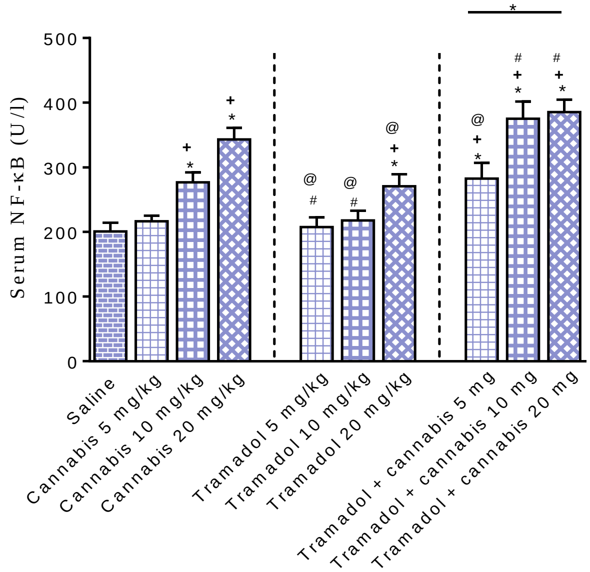

Figure 2 Nuclear factor-kappa B (NF-κB) in rat serum after treatment with different doses of cannabis, tramadol or cannabis/tramadol for 6 weeks. Data are mean ± SEM. *p < 0.05 vs. saline and between other groups as shown on the figure. +p < 0.05 vs. only cannabis 5 mg/kg, @p < 0.05 vs. cannabis 20 mg/kg, #p < 0.05 vs. tramadol 20 mg/kg

4. DISCUSSION

In the study, we provided evidence for an increase in the levels of NF-κB in both the brain tissue and serum of rats treated for 6 weeks with *Cannabis sativa* or tramadol, two widely abused substances. Several lines of evidence suggested a role for the transcription factor NF-κB and neuronal apoptosis or death in different brain pathologies. Increased expression and activation of NF-κB was found in after transient cerebral ischaemia (Stephenson et al., 2000), intracerebral hemorrhage (Hickenbottom et al., 1999) and after acute trauma to the brain (Wang et al., 2016) in the rat. The inactivation of NF-κB led to a decrease in cerebral edema after brain trauma (Wang et al., 2016) and the reduced expression of inflammatory chemokines and cytokines with better functional recovery after spinal cord contusion injury in the rat (Brambilla et al., 2005). Moreover, hypoxic-ischaemic brain damage in neonatal rats was markedly decreased by inhibiting the activity of cerebral NF-κB via a mechanism which involved inhibition of apoptosis (Nijboer et al., 2008).

There ample evidence to indicate a neurotoxic effect for cannabis or its major psychotropic component THC on brain function and structure (Abdel-Salam, 2022a, b). Cannabis impairs memory processing and this applied to working memory, semantic processing and retrieval performance (Solowij et al., 2002; Jager et al., 2006). Persistent cannabis usage in adolescents' results in poor academic functioning and achievement (Meier et al., 2015). Cannabis induces changes in brain structure, especially when usage beings at an early age (Battistella et al., 2014; Nader and Sanchez, 2018). Heavy users of cannabis are also likely to experience psychotic events or even schizophrenia in late life, with a possible genetic contribution (Di Forti et al., 2014; Hiemstra et al., 2018).

Animal research has provided more insight into the adverse effects of cannabis or THC on neuronal integrity. In rats, treatment with cannabis extracts with high content of Δ^9-THC for 4-6 weeks caused neuronal degeneration. Neurons appeared dark with decreased size of their nuclei. There were also neuronal vascuolations, apoptotic cells, brain cellular infiltrations and gliosis. These effects were dose-dependent (Abdel-Salam et al., 2014, 2020). In vitro, exposure of rat hippocampal neurons in culture to THC caused shrinkage of neuronal cell bodies, nuclear condensation and contraction as well as genomic DNA strand breaks (Chan et al., 1998). Increased caspase-3 expression was observed in rat cortical neurons treated with THC in vitro (Downer et al., 2001) and in cerebral cortex of after in vivo administration of cannabis in rats causes neuronal apoptosis evidenced by increased caspase-3 expression (Abdel-Salam et al., 2014). The precise mechanism by which cannabis or THC causes neuronal injury remains unclear but may involve brain inflammation, impaired mitochondrial biogenesis and activity and reduced brain energetic metabolism (Abdel-Salam, 2022a). In mice, THC caused the activation of cerebellar microglia and increased expression of interleukin-1β (IL-1β) (Cutando et al., 2013).

Glial fibrilar acidic protein (GFAP) is a marker of reactive gliosis whereby brain injury causes the activation of microglia and astroglia with increased expression of GFAP. Rats treated with THC showed increased GFAP staining in the hippocampus and parietal cortex (Lopez-Rodriguez et al., 2014). GFAP proteins were also increased in the brain and serum after cannabis administration in rats (Abdel-Salam et al., 2019). Microglia and astrocytes express CB1 and CB2 cannabinoid receptors (Stella, 2010). The activation of glia cells has been linked to neurodegeneration via the release of inflammatory cytokines, reactive oxygen metabolites (Phatnani and Maniatis, 2015). Rat cerebral mitochondria exposed to THC exhibited decreased mitochondrial coupling, increased H_2O_2 production and free radical leak (Wolff et al., 2015). In addition, decreased brain mitochondrial oxygen consumption and uncoupling of oxidative phosphorylation has been reported after treatment of rats with 10 mg/kg, twice daily for 4.5 days (Costa and Colleoni, 2000). We in addition have shown that the transcription factor, nuclear respiratory factor-2 (NRF-2) which is required for controlling mitochondrial respiratory function and oxidative phosphorylation is decreased in serum of cannabis treated rats (Abdel-Salam et al., 2020).

The effect of cannabis or its major psychotropic constituent THC on NF-κB is crucial in delineating the mechanisms underlying their neurotoxic effects. Pesche et al., (2008) found that inhibition of NF-κB expression stimulated by phorbol myristate acetate in cancer cell lines in vitro by THC, cannabidiol, cannabinol and cannabigerol to be almost negligible at their non-toxic concentrations. Another study showed that THC treatment of dendritic cells from murine bone marrow induced apoptosis (caspase-2, -8 and -9 activation, cleavage of Bid and cytochrome c release). THC induced phosphorylation of IκB-α and thereby, increased the transcription of a number of apoptotic genes that are regulated by κB whilst inhibition of the latter prevented apoptosis induced by THC in dendritic cells (Do et al., 2004). Our results extend the above studies and suggest a role for increased expression and activation of NF-κB in neuronal damage caused by THC or by cannabis rich in THC content. The present study also showed a significant increase in NF-κB in brain and serum by tramadol which suggest that NF-kB may play a role in the neurodegerative changes found in rat brain after high doses of the drug.

5. CONCLUSIONS

The present study provided the first evidence for increased brain and serum NF-κB in rats treated for 6 weeks with cannabis extract, tramadol or their combination. These observations suggest a role for brain inflammation induced by NF-κB activation in the development of neuronal damage by these drugs.

Author contribution

O.M.E.A.S. and E.R.Y. conducted the research and biochemical analysis. N.M.S. performed the histopathology study and its interpretation. O.M.E.A.S. wrote and prepared the manuscript, O.M.E.A.S., E.R.Y. and N.M.S. approved the final version of the manuscript.

Ethical approval

The experimental procedures were performed in compliance with the Institutional Ethics Committee and with the guidelines for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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