

Oxidative Stress and Neuronal Injury After Cannabis and Ketamine Administration

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Abstract: - Cannabis sativa and ketamine are common substances of abuse causing psychotic events and neurodegeneration. In this study, the effect of pretreatment with Cannabis sativa extract on oxidative stress, inflammatory mediators and brain damage induced by ketamine was investigated. Rats were treated with subcutaneous injections of cannabis extract (10, 20, 30 or 40 mg/kg; expressed as Δ^9 -THC content) daily for three weeks and then in combination with ketamine (15 mg/kg, intraperitoneally) for another 5 days. Rats were tested for biochemical markers of oxidative stress including malondialdehyde (MDA) reduced glutathione (GSH), and nitric oxide (NO) concentrations in brain. Paraoxonase-1 (PON-1) activity, and levels of the proinflammatory cytokines, interleukin-1 β (IL-1 β), and tumour necrosis factor- α (TNF- α) in brain were also determined at the end of treatment period. Results indicated that compared with the saline control group, ketamine induced significant elevation in brain MDA and NO, which was accompanied by depletion of GSH and inhibition of PON-1 activity. Ketamine also significantly increased brain IL-1 β and TNF- α and induced neuronal necrosis, apoptosis and vacuolation. Cannabis sativa (20-40 mg/kg) pretreated rats showed lower levels of oxidative stress and inflammation and doses of 30 or 40 mg/kg slightly reduced neuronal apoptosis and necrosis. These findings suggest that cannabis constituents do not enhance the neurotoxic effects of ketamine and might partly counteract the effects of ketamine-induced NMDA antagonism by reducing the release of free radicals and inflammatory mediators in brain.

Key-Words: - Cannabis sativa, ketamine; oxidative stress, neuroinflammation, neurodegeneration

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1 Introduction

Cannabis sativa is the most widely abused substance worldwide. It is commonly available forms are marijuana, from the plant leaves and flowers or hashish which is the compressed resin and flowers [1]. The popularity of *Cannabis sativa* as a recreational drug is reflected in the fact that the UN office for drug and crime estimated that in 2018, cannabis was used by ~192 million adults globally [2]. Cannabis is usually smoked in the form of cigarettes or water pipe. Users experience the so called "high"; euphoria or, relaxation and perceptual alterations [1]. The principal constituent responsible for the psychotropic effects of cannabis is delta-9-tetrahydrocannabinol [Δ^9 -THC] [3]. The effects of cannabis are the result of complex interaction between more than 100 different cannabinoid compounds that might have an antagonistic or

synergistic actions to that of Δ^9 -THC [4]. These cannabinoids and their endogenous legends or endocannabinoids exert their effects by acting on two G-protein coupled receptors; CB1 expressed in brain and CB2 expressed predominantly on immune cells in the periphery [5]. In this context, Δ^9 -THC, acts as a partial agonist at CB1 receptors [6]. Cannabis also contains many other compounds including terpenes, carbohydrates, fatty acids and their esters, phytosterols, and phenolic compounds [4]. Studies suggested an association between heavy and regular cannabis use and the development of psychosis [7]. Chronic heavy users also showed brain structural alterations [8] while animals studies demonstrated neurotoxic effects for cannabis in high doses [9,10].

Ketamine, a phencyclidine derivative, is a dissociative anesthetic with psychedelic and psychotic properties. The drug is a competitive antagonist at the *N*-methyl-D-aspartate (NMDA)

subtype of glutamate receptor subtype on GABA-ergic interneurons, resulting in increased release of glutamate in the prefrontal cortex [11]. It is used at subanesthetic doses for alleviating pain or inducing mild sedation in humans [12,13] and as an adjunct to morphine in the management of moderate to severe acute pain [14]. Because of its psychedelic properties, ketamine is a drug of abuse, causing dissociation, hallucinatory experiences and altered sensory perceptions [15]. It is also capable of inducing psychotic-like symptoms both in humans and rodents and therefore is widely used as an experimental model of schizophrenia [15,16]. The widespread use of ketamine as a recreational drug in recent years has raised concerns as regards possible health consequences. Frequent, daily use is associated with neuro-cognitive impairment, deficits in working and episodic memory and frontal lobe white matter abnormalities [11]. In rodents, ketamine induces neuronal degeneration both in developing [17] and adult [18] brain.

Cannabis is considered a gateway to other drugs of abuse and so users of cannabis will also use other psychotropic drugs making the combined abuse of cannabis and ketamine a likely possibility. Whether this will result in increased neurotoxicity is not known. The aim of this study was therefore to investigate whether the prior administration of *Cannabis sativa* is able to modulate the neurodegeneration induced by ketamine.

2 Materials and methods

2.1 Animals

Sprague–Dawley rats of both sexes, weighing 160–170 g, from the animal house colony of the National Research Centre (NRC) were used in the study and fed with standard laboratory chow and water ad libitum. Animal procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals (Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes).

2.2 Drugs and Chemicals

Cannabis sativa and ketamine were obtained from the Ministry of Justice. Other chemical and reagents were obtained from Sigma (St. Louis, USA). Ketamine was dissolved in saline to obtain the necessary doses.

2.2.1 Preparation of the cannabis extract

The extract of *Cannabis sativa* L. was obtained from the dried flowering tops and leaves of the plant by chloroform treatment according to Turner and Mahlberg [19] with modification. In brief, 10 g of dried cannabis was ground with a mortar and pestle. Cannabinoids are enzymatically biosynthesised in the plant as their corresponding carboxylic acid forms. Neutral cannabinoids are formed via decarboxylation of the acidic cannabinoids during exposure to heat [19]. Decarboxylation of the plant material was achieved by placing the sample in a glass test tube (30 mL) and covering it with aluminum foil. The test tubes were placed in boiling water bath (100 °C) for 2 h. Ten milliliters of analytical grade chloroform was added and allowed to react overnight. The dried cannabis was extracted three times and fractions were combined, filtered over filter paper and collected in 100 mL volumetric flask. The filtrate was evaporated under a gentle stream of nitrogen (on ice and protected from light and stored at 4 °C and protected from light in an aluminum-covered container, which provided the dry extract as residue. For injection, the residue was re-suspended in 2 mL of 96% ethanol and the total volume in the volumetric flask increased to 100 mL by adding distilled water. The extract was injected subcutaneously at doses of 5, 10, 30 and 40 mg/kg (expressed as Δ^9 -THC). Tetrahydrocannabinol (THC) content was quantified using “GC mass spec”. The dry extract contained 10% of the Δ^9 -THC.

2.3 Experimental design

Different groups of rats (n = 6 each) were used. Rats were randomly assigned to the following treatment groups:

Group 1 received the vehicle (0.2 ml saline) daily. Groups 2, 3, and 4 received cannabis (5, 10, 30 and 40 mg/kg; equivalent to the active constituent Δ^9 -tetrahydrocannabinol) subcutaneously daily for three weeks and then together with i.p. ketamine (15 mg/kg) for another 5 days. Group 5 received saline subcutaneously daily for three weeks and then together with i.p. ketamine (15 mg/kg) for another 5 days.

Rats were subsequently euthanized by decapitation. The whole brain of each rat was rapidly removed out and snap-frozen in liquid nitrogen. Brain tissue samples were stored at – 80 °C until further processing. Frozen samples were thawed and homogenized in a glass tube with a Teflon dounce pestle in ice-cold phosphate buffer solution (50 mM Tris-HCl, pH 7.4) and sonicated. Homogenized samples were then centrifuged at 9000 g for 5 min at

4 °C. The supernatant was stored at – 80 °C until further analysis. Biochemical markers of oxidative stress including GSH, MDA, nitric oxide, paraoxonase-1, and the proinflammatory cytokines IL-1 β , TNF- α in the brain were estimated at end of the treatment period. The histopathological study was carried out on separate groups of rats (n = 4 per group).

2.4 Biochemical assessments

2.4.1 Lipid peroxidation

Lipid peroxidation was measured by determination of malondialdehyde (MDA) in the brain homogenates [20]. Thiobarbituric acid reactive substances (TBAS) react with thiobarbituric acid forming TBA-MDA adduct and the absorbance is read at 532 nm using spectrophotometer.

2.4.2 Reduced glutathione

Reduced glutathione (GSH) was determined in homogenates according to Ellman [21]. Briefly, DTNB (5,5'-dithiobis (2-nitrobenzoic acid) or Ellman's reagent is reduced by the free sulfhydryl group on GSH molecule to generate 5-thio-2-nitrobenzoic acid which has yellow color and can be determined by reading absorbance at 412 nm.

2.4.3 Nitric oxide

Nitric oxide, estimated as nitrate/nitrite was determined using the Griess reaction. In this assay nitrate is converted to nitrite via nitrate reductase. Griess reagent then acts to convert nitrite to a deep purple azo compound that can be determined using spectrophotometer at 540 nm [22].

2.4.4 Paraoxonase-1

The arylesterase activity of PON-1 was determined by a colorimetric method using phenyl acetate as a substrate. In this assay, PON-1 catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol was measured by monitoring the increase in absorbance at 270 nm at 25°C. The working mix consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM CaCl₂ and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer were added to the above mix and the changes in absorbance were recorded following a 20 s lag time. One unit of arylesterase activity is equal to 1 μ mole of phenol formed per min. The PON-1 activity is expressed in kU/l, based on the extinction coefficient of phenol of 1310 M⁻¹cm⁻¹. Blank samples containing water were used to correct for the spontaneous hydrolysis of phenyl acetate [23].

2.4.5 Interleukin-1 β

IL-1 β was measured in tissue supernatants using commercially available IL-1 β ELISA kit (KOMA BIOTECH Inc.) according to manufacture instructions. The kit uses a double antibody sandwich enzyme linked immunosorbent assay to measure the level of IL-1 β .

2.4.6 Tumour necrosis factor- α

TNF- α levels in the supernatants were measured using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit supplied by Thermofischer Scientific Co, USA according to the manufacture instructions.

2.5 Histopathological assessments

The brains from the control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal sections of 5 μ m thickness. The sections were stained with Hemotoxylin and Eosin (Hx & E) dye for histopathological observations.

2.6 Statistical analysis

All data were expressed as the mean \pm SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used. Statistical significance was considered at a probability value of less than 0.05.

3 Results

3.1 Oxidative stress

3.1.1 Lipid peroxidation

In ketamine only-treated rats, MDA contents of cerebral cortex and striatum increased significantly by 56.3% and 45.0%, respectively, from saline control values 17.68 \pm 0.75 and 17.14 \pm 0.25 to 27.63 \pm 1.8 and 24.84 \pm 0.40 nmol/g.tissue. The cannabis/ketamine treated groups exhibited significantly lower MDA values compared with the ketamine only group. Cannabis given at 20, 30 and 40 mg/kg decreased MDA levels in cerebral cortex 15.5, 23.6 and 26.9%, respectively, from control levels of 27.63 \pm 1.8 to 23.36 \pm 0.64, 21.1 \pm 0.73 and 20.21 \pm 0.58 nmol/g.tissue. Meanwhile, MDA levels in striatum decreased by 12.3 and 22.1% by cannabis at 30 or 40 mg/kg, respectively (21.78 \pm 0.48 and 19.35 \pm 0.60 vs. 24.84 \pm 0.40 nmol/g.tissue) (Fig. 1).

3.1.2 Reduced glutathione

Ketamine significantly decreased GSH levels in cerebral cortex and striatum by 58.4% and 43.1%

from saline control levels of 3.94 ± 0.08 and 3.69 ± 0.1 to 1.64 ± 0.07 and $2.10 \pm 0.11 \mu\text{mol/g.tissue}$. In rats treated with 30 and 40 mg/kg cannabis, GSH levels were significantly increased by 45.7% and 74.4% in cerebral cortex (2.39 ± 0.15 and 2.86 ± 0.21 vs. $1.64 \pm 0.07 \mu\text{mol/g.tissue}$) and by 33.8% and 54.8% in striatum (2.81 ± 0.13 and 3.25 ± 0.17 vs. $2.10 \pm 0.11 \mu\text{mol/g.tissue}$), respectively (Fig. 1).

3.1.3 Nitric oxide

Compared with the saline control group, ketamine induced significant decrease in nitric oxide levels in cerebral cortex and striatum by 21.0% and 23.9%, respectively, from saline values 19.88 ± 0.64 and 18.78 ± 0.47 to 15.72 ± 0.43 and $14.30 \pm 0.12 \mu\text{mol/g.tissue}$. Cannabis given at 30 or 40 mg/kg resulted in further decrease in nitric oxide levels in cerebral cortex by 16.1% and 23% and in striatum by 9.6% and 15.4%, respectively (Fig. 1).

3.4 Paraoxonase-1

In the ketamine only treated group, PON-1 activity showed significant decrease by 38.9% in cortex (8.1 ± 0.27 vs. 13.25 ± 0.24 kU/l) and by 43.7% striatum (6.97 ± 0.19 vs. 12.38 ± 0.39) compared with the saline control group. In the cannabis/ketamine groups, PON-1 activity was significantly increased in cerebral cortex by 36.3%, 43.3%, 55.9% by cannabis given at 20, 30 or 40 mg/kg (11.04 ± 0.35 , 11.61 ± 0.24 , 12.63 ± 0.32 vs. 8.1 ± 0.27 kU/l). Significantly increased PON-1 activity by 26.7% and 45.0% was also observed in the striatum of rats treated with cannabis at 30 or 40 mg/kg (8.83 ± 0.38 , 10.1 ± 0.17 vs. 6.97 ± 0.19 kU/l) (Fig. 1).

3.5 Interleukin-1 β

A significant increase in IL-1 β level in cerebral cortex was observed in rats treated with only ketamine compared with their saline controls (157.3 ± 4.1 vs. 76.1 ± 2.2 pg/ml). On the other hand, rats pretreated with cannabis at 40 mg/kg showed significantly lower IL-1 β level by 14.0% compared with the ketamine control group (135.2 ± 6.9 vs. 157.3 ± 4.1 pg/ml) (Fig. 2).

3.6 Tumour necrosis factor- α

The level of TNF- α in cerebral cortex was significantly increased in the ketamine only group compared with the saline control (102 ± 3.1 vs. 40.1 ± 2.0 pg/ml). Cannabis given at 30 or 40 mg/kg prior to ketamine significantly decreased the raised TNF- α by 19.2 and 30.5%, respectively (82.4 ± 2.4 , 70.9 ± 1.8 vs. 102.0 ± 3.1 pg/ml) (Fig. 2).

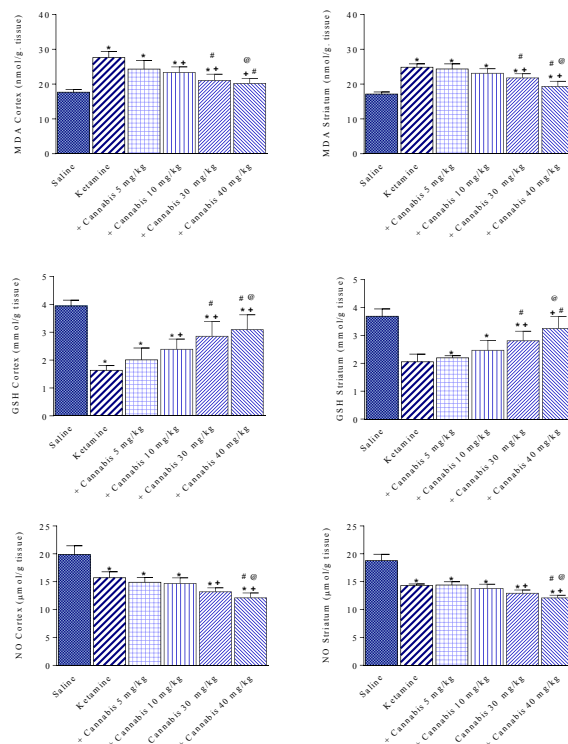


Fig. 1. Effect of ketamine or cannabis/ ketamine on brain malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO). Rats received s.c. injections of cannabis extract (10, 20, 30 or 40 mg/kg; expressed as Δ^9 -THC content) daily for three weeks and then in combination with ketamine (15 mg/kg, i.p.) for another 5 days. *: $P < 0.05$ vs. saline control. +: $P < 0.05$ vs. ketamine only group. #: $P < 0.05$ vs. ketamine/cannabis 5 mg/kg treated group. @: $P < 0.05$ vs. ketamine/cannabis 10 mg/kg treated group.

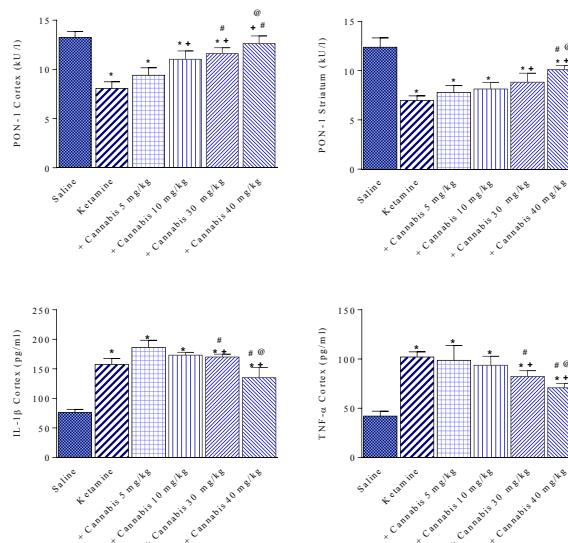


Fig. 2. Paraoxonase-1 (PON-1), interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) in the rat brain after treatment with saline, ketamine or cannabis/ ketamine. Rats received s.c. injections of cannabis extract (10, 20, 30 or 40 mg/kg; expressed as Δ^9 -THC content) daily for three weeks and then in combination with ketamine (15 mg/kg, i.p.) for another 5 days. *: P<0.05 vs. saline control. +: P<0.05 vs. ketamine only group. #: P<0.05 vs. ketamine/cannabis 5 mg/kg treated group. @: P<0.05 vs. ketamine/cannabis 10 mg/kg treated group.

3.7 Histopathological results

Sections from the control group showed normal histological structure of the cerebral cortex and striatum (Fig. 3A & 4A). The group treated with ketamine at 15 mg/kg for 5 days showed numerous histopathological changes including a large number of damaged neurons, degenerated, necrotic and vacuolated cells. Nuclei of the cells were shrunken, pyknotic and congestion of cerebral blood vessel was seen. Also many of damaged astrocytes were in the form of pyknotic, shrunken and vacuolated (Fig. 3B & 4B). In the groups treated with cannabis for 3 weeks prior to ketamine administration, moderately to mild amount of necrosis, vacuolated, pyknotic, apoptotic neurons and mild congestion of cerebral blood vessel were observed in the cortex and striatum, but the mild damage was observed at high doses of cannabis (30 & 40 mg/kg) as compared to the group treated with ketamine alone (Fig. 3C-F & Fig. 4C-F). Cannabis at high doses given prior to ketamine therefore was associated with less damage and only marginally alleviated ketamine -induced neurotoxicity.

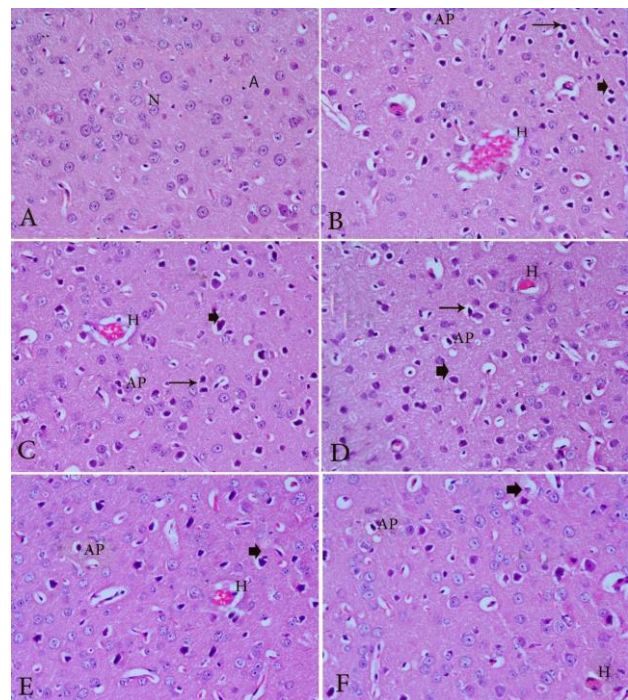


Fig. 3. Representative Hx & E stained cerebral cortex sections after treatment with: (A) Saline: showing normal histological structure of cortex with normal neurons (N) and astrocytes (A). (B) Ketamine alone showing degenerated, necrosis, vacuolation of the neurons shrunken, pyknotic (arrowhead), apoptotic nuclei (AP) and congestion of cerebral blood vessel (H) vacuolated and pyknotic astrocytes (thick arrow). (C) Cannabis 5 mg/kg + ketamine: showing moderate degeneration, necrosis and vacuolation of neurons, shrunken, pyknotic (arrowhead), and apoptotic nuclei (AP) and congestion of cerebral blood vessel (H) as well as vacuolated and pyknotic astrocytes (thick arrow). (D) Cannabis 10 mg/kg + ketamine: showing moderate degeneration, necrosis and vacuolation of neurons, shrunken, pyknotic (arrowhead), apoptotic nuclei (AP), congestion of cerebral blood vessel (H) and vacuolated and pyknotic astrocytes (thick arrow). (E&F) Cannabis 30 and 40 mg/kg + ketamine: showing mild degeneration, necrosis and vacuolation of the neurons, shrunken, pyknotic (arrowhead), apoptotic nuclei (AP) and congestion of cerebral blood vessel (H) (Hx & E \times 400).

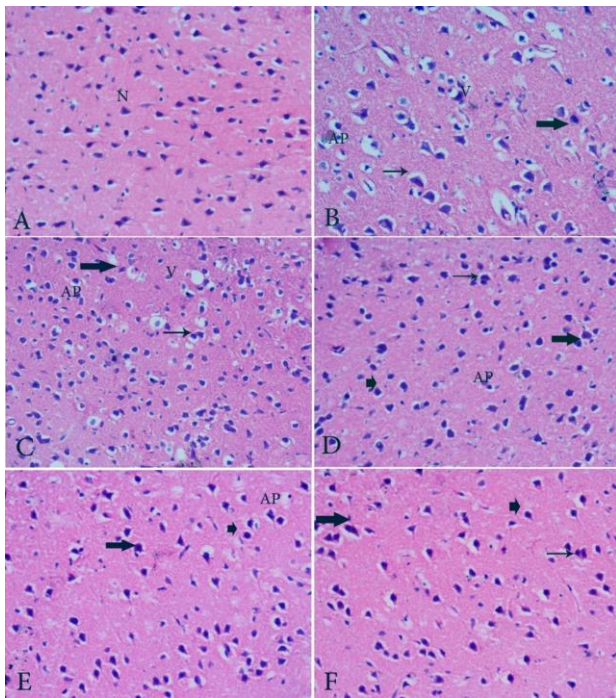


Fig. 4. Representative Hx & E stained sections of rat striatum after treatment with: (A) Saline: normal histological structure with normal neurons (N). (B) Ketamine alone showing degenerated, necrotic, vacuolated (V) neurons, shrunken, pyknotic nuclei (thin arrow), apoptotic cells (AP) and pyknotic astrocytes (thick arrow). (C & D) Cannabis 5 & 10 mg/kg + ketamine: showing moderate degeneration, necrosis, vacuolation of neurons (V), shrunken, pyknotic nuclei (thin arrow), associated apoptotic cells (AP) and pyknotic astrocytes (thick arrow). (E & F) Cannabis 30 and 40 mg/kg + ketamine: showing mild degeneration, necrosis of neurons, pyknotic nuclei (thin arrow), apoptotic cells (AP) and pyknotic astrocytes (thick arrow) (Hx & E $\times 400$).

3 Discussion

Cannabis sativa and ketamine, two commonly abused substances, have been associated with the development of psychotic events and neurotoxicity. In the current study, we investigated the effect of Cannabis sativa on brain oxidative stress, neuroinflammation and neurodegeneration induced by ketamine injection in the rat. Our results show that ketamine given for 5 consecutive days to rats caused brain oxidative stress. Furthermore, brain levels of IL-1 β and TNF- α were upregulated. Ketamine induced neuronal atrophy with loss of prominent axons in cortex and striatum. The prior administration of Cannabis sativa extract for three weeks reduced ketamine-induced oxidative stress, the elevations in IL-1 β and TNF- α . Moreover, there were reduced neuronal apoptosis and necrosis.

The effect of ketamine on oxidative stress reported herein is also supported by a number of studies. Increased lipid peroxidation and protein carbonyl content in prefrontal cortex was shown after a single dose of ketamine (15 or 25 mg/kg, i.p.) in adult rats [24] and mice [25]. Ketamine given to mice at 30 mg/kg, i.p., for two consecutive days, was shown to increase IL-6 production, activate NADPH oxidase, increase brain superoxide and cause neuronal injury of a subset of interneurons in prefrontal cortex [26]. There was increased staining for the DNA oxidation marker 8-OHdG in the prefrontal cortex of mice treated with ketamine (30 mg/kg, i.p.) [27]. Increased reactive oxygen species (ROS) formation in mitochondria, reduced mitochondrial membrane potential, mitochondrial swelling and cytochrome c release in mitochondria were observed following ketamine in adult rats (30 mg/Kg daily for five consecutive days) [28]. We have also demonstrated increased lipid peroxidation (MDA), and decreased GSH in cerebral cortex and striatum of mice receiving a single dose of ketamine (15 or 30 mg/kg, s.c.) [18]. Oxidative stress therefore is evident in rodent brain after ketamine injection and that make free radical mediated mechanisms an important contributor to the neurodegeneration observed in these animals.

The present study in addition demonstrated a significant inhibition PON1 activity in the brain of ketamine-treated rats which confirms our earlier observations [18,29]. The activity of this enzyme which possesses anti-oxidative and anti-inflammatory properties decreases in serum of patients with a number of neurological disorders in which free radical-mediated oxidative injury has been implicated in their pathogenesis [30-32]. Increased oxidative stress is found in patients with psychosis [33] and in untreated patients with first-episode psychosis, serum PON-1 activity is also decreased and this decrease correlated with an increase in proinflammatory cytokines [34]. The enzyme is inactivated by high levels of oxidative stress [35], which could explain a decrease in its activity in neurological disorders as well as in the current study.

We also showed that ketamine increased brain levels of IL-1 β and TNF- α . A previous study found that a single dose of ketamine (15 or 30 mg/kg, s.c.) increased TNF- α in cortex in both saline and LPS-treated rats [18]. Sedky et al. [36] reported increased level of TNF- α in brain of rats by 25 mg/kg of ketamine (i.p., for one week) while Abelaira et al.

[24] showed increased TNF- α in serum of rats given a single i.p., dose of 15 mg/kg. Moreover, a study by Li et al. [37] reported increased levels of IL-1 β in rat hippocampus after single and repeated doses of ketamine. TNF- α , however, increased after single dose of ketamine but decreased following repeated ketamine injections. These proinflammatory cytokines IL-1 β and TNF- α are capable of exacerbating neurodegeneration [38,39] and an increase in their levels could underlie the neuronal injury caused by ketamine. Other studies, however, reported inhibition of proinflammatory cytokines by ketamine both in vitro [40] and in vivo [41]. Thus, ketamine at 10 mg/kg, i.p., for two days was found to decrease the expression of IL-6 and IL-1 β in rat prefrontal cortex and hippocampus [41]. The discrepancies in the effect of ketamine on proinflammatory cytokines are probably caused by different doses and/or the experimental models used.

Consistent with previous reports [18,29,42,43] ketamine induced neuronal injury. Ketamine increased cell death in hippocampal CA3 of adult mice [42]. In neurons from human embryonic stem cells (hESCs) ketamine increased ROS production and cytochrome c release from mitochondria, induced ultrastructural abnormalities and caused cell death [43]. In humans, cortical atrophy has been reported in chronic users of the drug [44]. The neuropharmacological effects of ketamine are complex. Ketamine effects are mainly attributed to blockade of NMDA glutamate type receptors [45]. Glutamate is the major excitatory neurotransmitter in the CNS [46]. Ketamine has been shown to enhance the release of glutamate in cortex and striatum [47,48], decreases GABA-ergic inhibitory neurotransmission [49] and increases neuronal cortical excitability [48]. It has also been shown that prolonged exposure to the drug increases NMDA receptor expression, resulting in higher/toxic influx of calcium into neurons, increased ROS generation and neuronal cell death [50]. Excitotoxicity and/or free radical-mediated oxidative stress thus might underlie the neurotoxicity of ketamine.

The present study suggests that the pretreatment with Cannabis sativa reduces the ketamine effects on oxidative stress, neuroinflammation and neuronal injury. In fact, these findings were unexpected in view of experimental [9,10,51,52] and human data [8] suggestive of neurodegenerative effect for long-term and heavy cannabis use. Although in this study we did not investigate the effect of cannabis alone, previous studies showed that treatment of rats with Cannabis sativa at doses of 10 or 20 mg/kg once a

day s.c., for 2-6 weeks was associated with dose-dependent signs of neurodegeneration in the form of dark and shrunken nuclei, dark apoptotic neurons, focal gliosis as well as increased caspase-3 immunoreactivity [9,10, 53]. Cannabis also increased neuronal damage in hippocampus and cerebral cortex and caspase-3 immunoreactivity in degenerating neurons of the cortex after LPS injection in mice [53]. Other researchers reported shrinkage of neuronal cell bodies and nuclei besides genomic DNA strand breaks in hippocampal neurons exposed to THC [51]. Cannabis did not reduce nigrostriatal damage in mice treated with rotenone and/or LPS despite lowered levels of oxidative stress [53]. Cannabis given before inducing global cerebral ischaemia, however, conferred protection against the ischaemic neuronal injury [54]. In cerebral ischaemia, there is excessive release of glutamate and over-stimulation of NMDA, AMPA and kainate receptors, resulting in intracellular accumulation of toxic levels of calcium and neuronal death [55]. Δ^9 -THC or other cannabinoids have been shown to prevent neuronal death in models of glutamate-induced excitotoxic neuronal injury [56,57]. Δ^9 -THC and cannabinoid receptor agonists act presynaptically to inhibit the release of glutamate, and excitatory synaptic transmission [58,59]. Cannabinoids also counteract the toxic effects of glutamate-NMDA on stimulation of the nitric oxide and zinc release [60]. Cannabis and/or Δ^9 -THC by affecting glutamate release and reducing excitotoxicity might thus counteract the effect of ketamine on neuronal integrity. Other mechanisms including inhibition of TNF- α , IL-1 β and NO release by glia cells, and decreased oxidative stress by cannabis constituents could also be involved.

5 Conclusion

In summary, this study showed for the first time that a Cannabis sativa extract rich in Δ^9 -THC given as a pretreatment to ketamine-treated rats was associated with reduced neuronal damage compared with ketamine only treatment. This might be due to blockade of ketamine effect in the presence of some compounds in the cannabis. Clearly, further studies are needed to delineate the effects of prolonged cannabis use on neurodegeneration and cognitive performances induced by chronic ketamine use.

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