Comparative Clinical Pathology (2019) 28:1229–1236
https://doi.org/10.1007/s00580-018-2847-6

ORIGINAL ARTICLE

Co-administration of nicotine ameliorates cannabis-induced behavioral deficits in normal rats: role of oxidative stress and inflammation

Mayada A. El-Hiny[1] · Dalaal M. Abdallah[2] · Omar M. E. Abdel-Salam[1] · Neveen A. Salem[1] · Zakaria A. El-Khyat[3] · Sanaa A. Kenawy[2]

Received: 7 August 2018 / Accepted: 25 October 2018 / Published online: 23 November 2018

Abstract

Nicotine (Nic) and cannabis are considered to be the most abused drugs worldwide that are progressively taken concomitantly. The present study aimed to investigate the modulatory effect of Nic on cannabis extract–induced neuro-inflammation, oxidative status, and the associated behavioral/biochemical alterations. Nic (0.25 mg/kg) and/or cannabis extract expressed as Δ^9-tetrahydrocannabinol (THC10/20; 10 and 20 mg/kg) were given intraperitoneally for 30 days to Wistar rats. Nic shortened the floating time in forced swimming test, increased locomotion in the open field test, and decreased escape latency in the Morris water maze when co-administered with THC. These effects were associated with the inhibition of THC-mediated elevations in brain interleukin-1 beta, lipid peroxidation, superoxide dismutase, and ascorbic acid. Additionally, Nic increased serum butyrylcholinesterase (BChE) when combined with THC without affecting the serum acetylcholinesterase enzyme. The combinations spiked the brain glucose content above normal. In conclusion, the co-administration of Nic reduced THC-induced depressive-like behavior and memory impairment as well as hypo-locomotion associated with THC20. Such effects could be linked to Nic-mediated inhibition of brain oxidative stress, inflammation, and decreased serum BChE deactivity.

Keywords Cannabis resin extract · Δ^9-tetrahydrocannabinol · Nicotine · Rats · Oxidative stress · Inflammation · Memory · Locomotion, depression

Introduction

Δ^9-tetrahydrocannabinol (THC) is the chief psychoactive constituent in the plant *Cannabis sativa* family *Cannabaceae* L which is linked to various health hazards (Volkow et al. 2014). According to the United Nations (2018), about 192.2 million people, which is about 3.9% of the world population, consume cannabis that makes it the most exceedingly abused illicit drug. THC manifests short- and long-term, physical and mental effects including impaired movement, memory, and attention as well as increased heart rate, depression, and anxiety (The National Academies of Sciences 2017). This is achieved by acting on specific cannabinoid receptors (CBRs) mainly CBR1 and CBR2. CBR1 plays a main part in the psychoactive and behavioral effects of cannabis (Tai and Fantegrossi 2014). It is mainly found centrally concentrated in the hippocampus which is involved in the memory and the cerebral cortex for cognition (Manzanares et al. 2006). CBR1 is also activated in the amygdala that is responsible for emotional responses. In addition, it is found in the limbic forebrain and cerebellum which are involved in motivation and motor coordination, respectively (Iversen 2012; Hu and Mackie 2015). On the other hand, CBR2 is primarily found peripherally in the immune system (Iversen 2012) and also in the gastrointestinal tract, heart, skin, and reproductive organs (Madras 2015).

Nicotine (Nic) is a naturally occurring alkaloid found primarily in the plant *Nicotianatabacum*, family *Solanaceae* L. It causes every year more than 7 million deaths globally; of which, 6 million are the result of the direct use of tobacco, while 890,000 are second-hand smokers (WHO 2018). Nicotine acts on the nicotinic acetylcholine receptor (nAChR) which resides both peripherally and centrally

✉ Mayada A. El-Hiny
mayada_elhiny@hotmail.com

[1] Department of Toxicology and Narcotics, National Research Centre (NRC), Cairo, Egypt

[2] Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

[3] Medical Biochemistry Department, NRC, Cairo, Egypt

(Gotti and Clementi 2004). It has inverse dose-dependent protective antioxidant (Tunez et al. 2010) and deleterious prooxidant effects (Guan et al. 2003). Indeed, high Nic doses cause neurodegeneration via oxidative stress (Guan et al. 2003), while at a low-dose level, an anti-inflammatory effect through binding to neuronal α7 nAChRs is exerted (Viveros et al. 2006).

Cannabis and Nic are increasingly taken in combination, as Nic reduces the sedative effect of cannabis and increases and prolongs its rewarding effect (Tullis et al. 2003). Both induce complex dose-response effects, thus manifesting functional interactions in modulating the brain neurochemistry and behavioral aspects (Valjent et al. 2002). At the cellular level, CB1R and nAChRs are densely co-localized in the hippocampus to be involved in varied sets of modulatory processes (Filbey et al. 2015). Consequently, the current study investigated the potential modulatory effects of 30-days Nic coadministration with cannabis extract (10% THC) expressed as THC with emphases on the brain oxidative stress and inflammation status as well as behavioral outcomes in normal rats.

Material and methods

Animals

Adult male Wistar rats (150–200 g) were obtained from the animal house colony in the National Research Center (NRC; Giza, Egypt). Throughout the period of the investigation, the animals were housed in an ambient atmosphere, constant light cycle (12 h light/dark), and kept on a standard diet and tap water ad libitum.

Cannabis resin sampling and extraction

The dried cannabis resin sample (8,092,009/Azbakeyya Criminalistics) used in the current study was obtained from the Forensic Sciences of Ministry of Justice Laboratory (Cairo, Egypt). The chloroform extract was prepared with the modification previously described by Turner and Mahlberg (1984) at the laboratory of Toxicology and Narcotics Department (NRC, Cairo, Egypt). The dry extract was suspended in ethanol-saline (2%), and HPLC (Fischedick et al. 2009) quantification revealed that the mixture contained 10% THC.

Experimental design

Rats were classified into 6 groups, each included 9 rats that were daily injected intraperitoneally for 30 consecutive days with the tested compounds dissolved in saline. Rats that received saline served as the normal group, while those receiving 0.25 mg/kg Nic (File et al. 1998; Sigma-Aldrich, MO, USA) or THC (10 and 20 mg/kg; Pagotto et al. 2006) were designated as single-treatment regimen groups. Additionally, in the other 2 groups, the rats were injected by either Nic with THC10 or THC20.

Behavioral tests

Open field, forced swimming, and Morris water maze (MWM) tests were performed at the end of the experimental period to assess motor coordination and exploratory behaviors as well as depression and memory task.

Open field test

The open field test (Simonin et al. 1998) was carried out in a white squared wooden arena (80 cm wide, 80 cm long, and 40 cm high), with red walls divided by black lines into a total of 16 equal squares on the white floor. The test was performed under white light in a quiet room to avoid any external stimuli. The behaviors were recorded during an observation period of 5 min which included ambulation, rearing, and grooming frequencies.

Forced swimming test

In the forced swimming test, a 10-min pretest was done 24 h prior to the test day. The rats then were forced to swim individually in a plastic cylinder (46 cm in height with a 21-cm internal diameter), filled with water (25 °C) to a depth of 30 cm from which there was no escape. The passive (immobility) behavior was recorded for 5 min for each rat (Slattery and Cryan 2012).

Morris water maze test

Typically, the maze consists of a large pool with side walls of the 70 × 40 × 20 cm dimensions. The transparent platform's top surface was hidden 1 cm below the water surface and was made rigid to make it easy to climb on. After 3 days of training, for evaluating memory performance, the time taken to reach the platform was recorded for each rat (Graziano et al. 2003).

Preparation of samples

After 24 h from the last dose administration, rats were deeply anesthetized for blood collection and euthanized by cervical dislocation to excise the brains for homogenization in ice-cold 0.1 M phosphate buffer saline at pH 7.4.

Determination of brain oxidative stress biomarkers

Brain oxidative stress biomarkers were assessed in brain homogenates by determining malondialdehyde (MDA), ascorbic acid, and superoxide dismutase (SOD). MDA was determined by measuring thiobarbituric reactive species using the method described by Ruiz-Larrea et al. (1994) in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red-colored complex having a peak absorbance at 532 nm. The main principle of ascorbic acid methodology (Harris and Ray 1935) is the redox reaction of ascorbate with 2,6-dichlorophenolindophenol, where ascorbate is oxidized to dehydroascorbate, while 2,6-dichlorophenol indophenol is reduced to a colorless leuco base. SOD is determined using a commercially available kit, colorimetric (Biodiagnostic, Cairo, Egypt), according to the method described by Nishikimi et al. (1972).

Determination of brain interleukin 1-beta

Brain interleukin (IL)-1β was determined using a commercially available ELISA kit (Sun Red Biological Technology, Shanghai, PRC), according to the manufacturer's protocol.

Determination of butyrylcholinesterase and acetylcholinesterase activities

Serum butyrylcholinesterase (BChE; Knedel and Bottger 1967) activity was assessed depending on the hydrolysis of butyrylthiocholine by butyrylcholinesterase to give thiocholine and butyrate. A second reaction occurs between the resultant thiocholine and 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB), yielding 2-nitro-5-mercaptobenzoate (a yellow compound) which can be measured at 405 nm. The determination of acetylcholinesterase activity in serum is a modification of the Ellman et al. (1961) method as described by Gorun et al. (1978). The principle depended on the measurement of thiocholine produced upon the hydrolysis of acetyl thiocholine where the color was read immediately at 412 nm.

Determination of brain glucose content

Brain glucose content (Trinder 1969) was assessed by its conversion to peroxide and gluconic acid in the presence of glucose oxidase. The produced hydrogen peroxide reacted with phenol and 4-aminoantipyrine in the presence of peroxidase to yield a colored quinonemine which is measured spectrophotometrically at 510 nm.

Statistical analysis

Data were presented as means ± standard error of the means (SEM) or median (minimum–maximum). Comparison between groups was carried out using the parametric one-way ANOVA test followed by Tukey multiple comparison test except for the open field test where nonparametric Kruskal-Wallis one-way ANOVA was carried out followed by Dunn multiple comparison test. Differences were considered significant when $P < 0.05$. Graph-pad prism 6.00 for Windows software (CA, USA) was used to plot graphs and carry out these statistical tests.

Results

Nic improves locomotion in cannabis-treated rats

Nic-treated animals tended to show an increase in ambulation (Fig. 1a), rearing (Fig. 1b), and grooming (Fig. 1c) in comparison to the normal group in the open field test. On the other hand, THC10 administered alone reduced rearing by 93% relative to the normal group, while the co-administration of THC10 with Nic decreased ambulation, rearing, and grooming frequencies by 84, 85, and 68%, respectively, relative to Nic. However, Nic/THC20 increased ambulation and grooming by 5.2- and 7.7-folds, respectively, relative to THC20.

Nic reduces depressive-like behavior in cannabis-treated rats

Animals receiving THC20 showed an increase in floating time by 67% relative to the normal group while it was decreased by 44% (THC10) and 31% (THC20) in THC/Nic combinations relative to the corresponding cannabinoid dose. Notably, THC20/Nic increased the floating time by 130% relative to Nic0.25 group (Fig. 2).

Nic improves memory in cannabis-treated rats

In the MWM test, THC10 experienced increased latency by 62% relative to the normal group, while the administration of Nic/THC10 decreased it by 45% relative to the corresponding dose of THC10 (Fig. 3).

Nic decreases cannabis-induced oxidative stress

THC10 elevated MDA by 47% (Fig. 4a), where the addition of Nic decreased lipid peroxidation by 25% (Nic/THC10) relative to THC10 and 43% (Nic/THC20) compared to Nic. Tough THC10 only elevated ascorbic acid by 124% (Fig. 4b); in THC20-treated animals, both ascorbic acid (34%; Fig. 4b) and SOD (172%; Fig. 4c) contents were raised relative to that of the normal group. The co-administration of Nic with THC10 suppressed ascorbic acid (69%) relative to THC10,

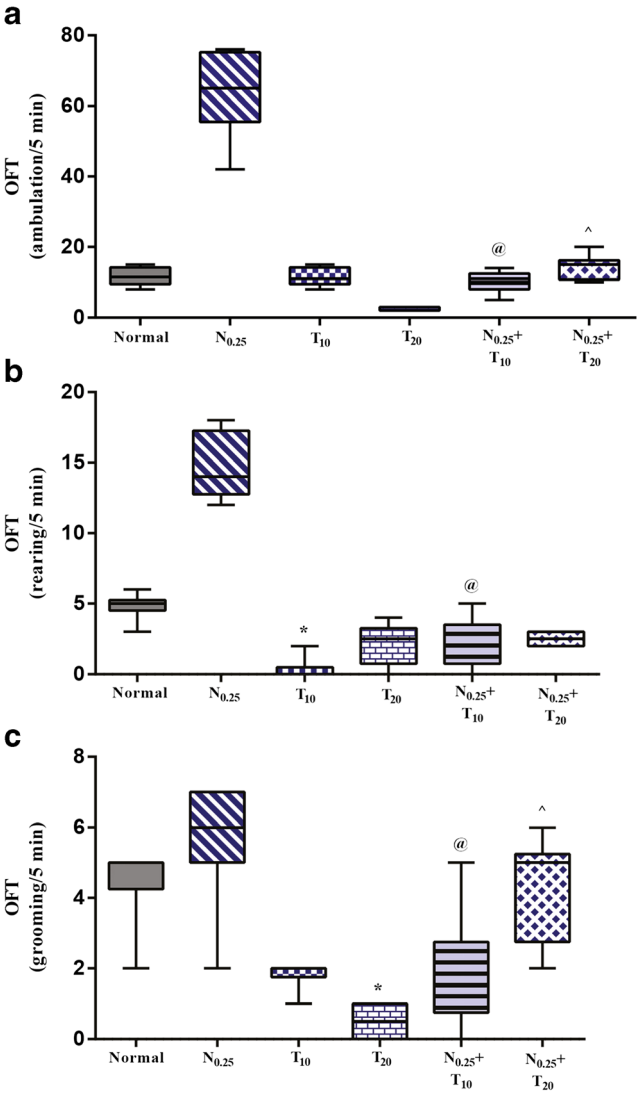

Fig. 1 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on ambulation (**a**), rearing (**b**), and grooming (**c**) frequencies in open field test in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. OFT was carried out on the 30th day of treatments. Results ($n = 6$ rats, per group) are expressed as boxplots presented with median (minimum–maximum) as well as 25th and 75th percentile values of ambulation (Fig. 1a), rearing (Fig. 1b), and grooming (Fig. 1c) frequencies among groups. Statistical analysis was carried out by nonparametric Kruskal-Wallis one-way ANOVA followed by Dunn multiple comparison test. $^{*,@,\wedge}$ $P < 0.05$, compared to normal, N0.25, and T20 groups respectively. OFT, open field test

an effect that was lower than both THC10-treated and normal rats. Meanwhile, Nic co-administered to normal rats receiving THC reduced the brain SOD content by 58% (Nic/THC10) and 74% (Nic/THC20) compared to their counter partners as well as to normal rats and their respective single regimens. Notably, SOD in Nic/THC20-treated animals was higher (53%) than Nic alone.

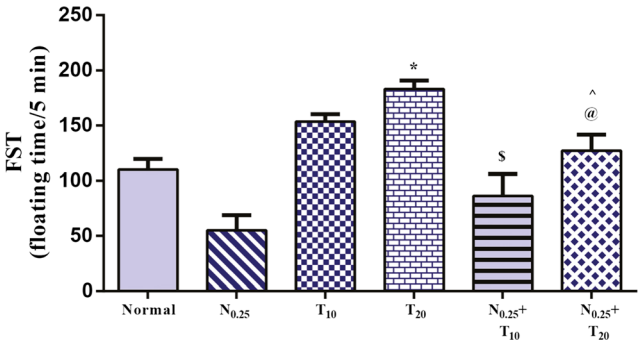

Fig. 2 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on forced swimming test in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. FST was carried out on the 30th day of treatments. Results are expressed as mean ± SEM ($n = 6$ rats). Statistical analysis was carried out by one-way ANOVA followed by Tukey multiple comparison test. $^{*,@,\$,\wedge}$ $P < 0.05$, compared to normal, N0.25, and T10/20 groups respectively. FST, forced swimming test

Nic decreases cannabis-induced inflammation

Notably, THC10/20 increased IL-1β to reach 2.5- and 1.7-folds in comparison to the control, respectively (Fig. 5). These values were leveled off to 39% (THC10/Nic) and 36% (THC20/Nic) when co-administered with Nic in comparison to THC10 and THC20, respectively.

Nic prevents cannabis-mediated serum BChE reduction and elevates brain glucose

Though all treatments did not alter serum AChE activity, THC10/20 suppressed BChE activity by 49 and 47%, respectively, when compared to the normal group, while Nic was

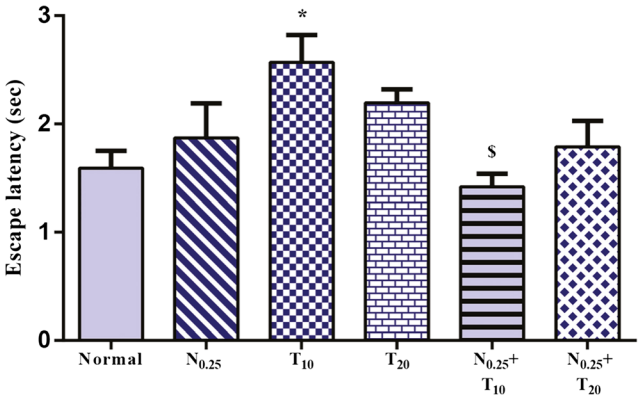

Fig. 3 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on escape latency on Morris water maze test in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. Morris water maze test was carried out on the 30th day of treatments. Results are expressed as mean ± SEM ($n = 6$ rats). Statistical analysis was carried out by one-way ANOVA followed by Tukey multiple comparison test. $^{*,\$}$ $P < 0.05$, compared to normal and T10 groups, respectively. MWM, Morris water maze test

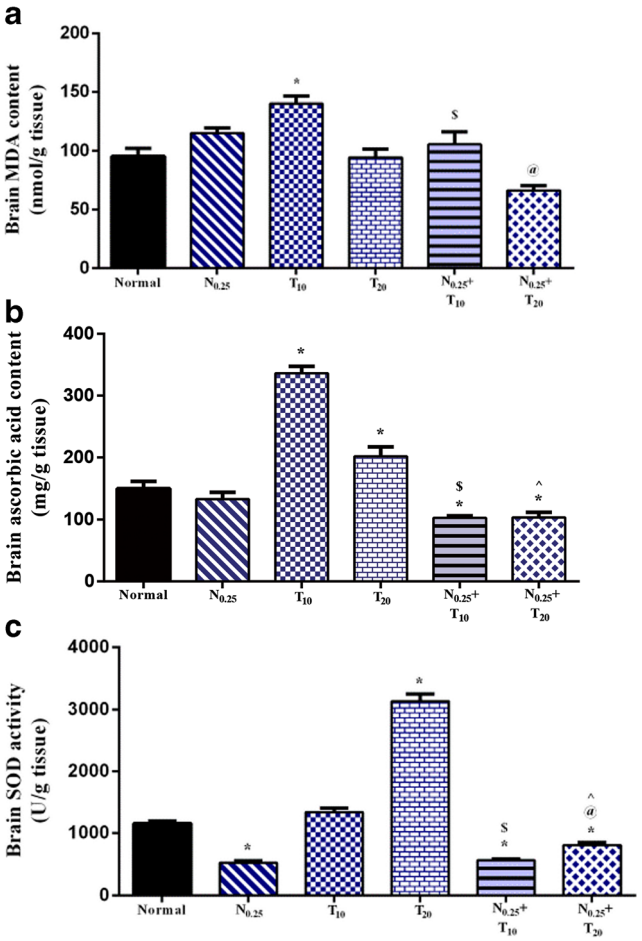

Fig. 4 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on brain malondialdehyde (**a**), ascorbic acid (**b**) contents, and superoxide dismutase activity (**c**) in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. Results are expressed as mean ± SEM ($n = 6$ rats). Statistical analysis was carried out by one-way ANOVA followed by Tukey multiple comparison test. $*,@,\$,\wedge$ $P < 0.05$, compared to normal, N0.25, and T10/20 groups, respectively. MDA, malondialdehyde; SOD, superoxide dismutase

able to normalize its level when combined with both cannabinoid doses (Fig. 6). Finally, although serum glucose was not changed, Nic/THC combination elevated brain glucose level relative to normal, Nic, and/or THC (Fig. 7).

Discussion

The present study clearly indicated that Nic co-administration with THC counteracted depression, memory deficits, and hypo-locomotion induced by THC in naïve rats. Nic co-treatment also decreased THC-induced brain oxidative stress and neuro-inflammation.

The administration of 10 mg/kg THC elevated lipid peroxidation and ascorbic acid in the brains of naïve rats. The redox imbalance could be explained by THC-induced oxidative stress that goes in line with a previous report (López-Malo

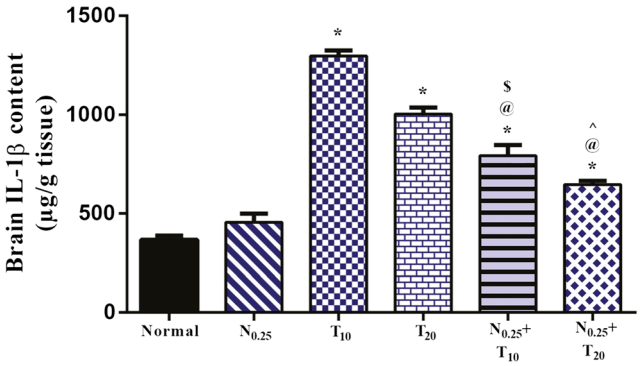

Fig. 5 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on brain interleukin-1β content in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. Results are expressed as mean ± SEM ($n = 6$ rats). Statistical analysis was carried out by one-way ANOVA followed by Tukey multiple comparison test. $*,@,\$,\wedge$ $P < 0.05$, compared to normal, N0.25, and T10/20 groups, respectively. IL-1β, interleukin-1 beta

et al. 2016). However, by doubling the THC dose, only ascorbic acid and SOD were increased. Herein, implying that the increased antioxidant defenses might have neutralized the free radicals that mediated the damage of the lipid cell membrane

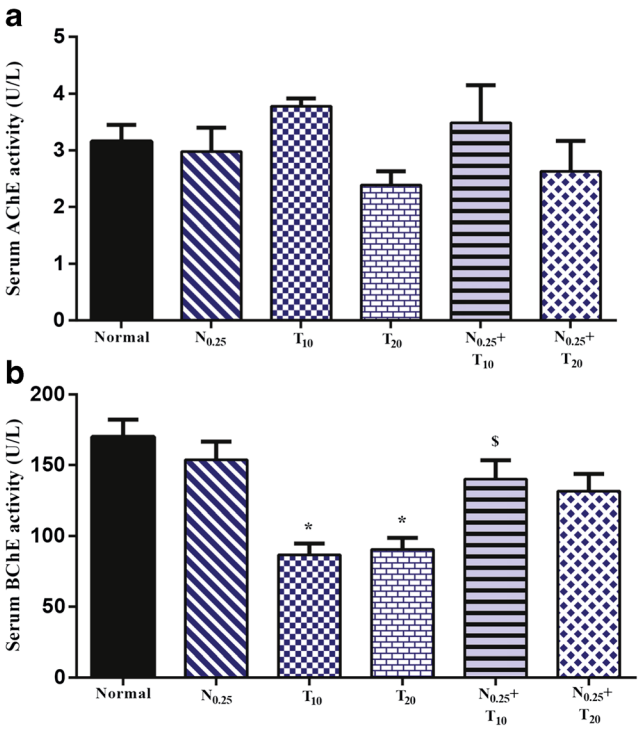

Fig. 6 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on serum acetylcholinesterase (**a**) and butyrylcholinesterase (**b**) activity in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. Results are expressed as mean ± SEM ($n = 6$ rats). Statistical analysis was carried out by one-way ANOVA followed by Tukey multiple comparison test. $*,\$$ $P < 0.05$, compared to normal and T10 groups, respectively. AChE, acetylcholinesterase; BChE, butyrylcholinesterase

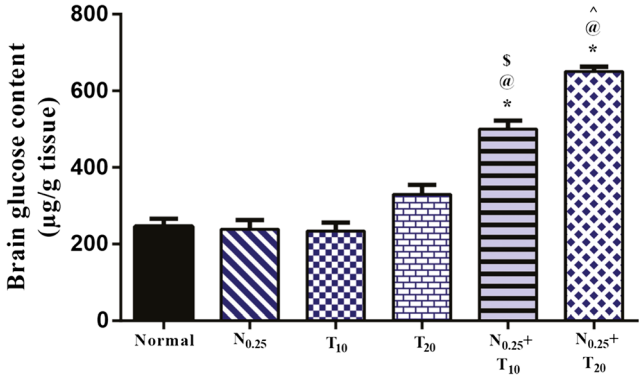

Fig. 7 Effects of 30-day nicotine and/or tetrahydrocannabinol administration on brain glucose content in rats. Rats were intraperitoneally injected daily with nicotine (N; 0.25 mg/kg) and/or tetrahydrocannabinol (T; 10 and 20 mg/kg) for 30 days. Results are expressed as mean ± SEM ($n = 6$ rats). Statistical analysis was carried out by one-way ANOVA followed by Tukey multiple comparison test. $^{*,@,\$,\hat{}}$ $P < 0.05$, compared to normal, N0.25, and T10/20 groups, respectively

was consistent with the study of Wolff et al. 2015. Free radicals, including reactive oxygen species (ROS), had been previously reported to be implicated in several behavioral changes (Tanasawet et al. 2017). These include depression, memory decline, and locomotion deficits (Degenhardt et al. 2002; Schoeler and Bhattacharyya 2013; Freedland et al. 2002). Accordingly, it could be speculated that the oxidative stress associated by THC consumption could be the culprit for the behavioral changes seen in the present work following the cannabis extract administration.

At the molecular level, the depression, cognitive, and locomotion impairments were depicted to be the consequences of CB1R activation by THC (Iversen 2003). Of note, the activation of the CBR is known to stimulate the transcriptional activation of nuclear factor (NF) κB (Jean-Gilles et al. 2015), which initiates the production of ROS via the activation of NADPH oxidase and the inflammatory cytokine IL-1β (Sedeek et al. 2013; Chen et al. 2015); both of which increase the transcriptional activity of NFκB again to amplify their production (Kaushal and Bansal 2014). Indeed, in this investigation, THC was also able to increase IL-1β in the brains of rats indicating the development of neuro-inflammation that plays an essential role in depression, memory loss (Bevan-Jones et al. 2017), and hypo-locomotion (Bonsall et al. 2015). The effect of the cannabinoid extract on the cytokine was supported by previous data (Cabral 2001). Henceforth, the inhibition of oxidative and inflammatory processes was likely implicated in the treatment of memory deficits, depression (Kruk-Slomka et al. 2016), and hypo-locomotion (Patel et al. 2016), where co-treatment with Nic, in the present study, could provide therapeutic benefits against the reported behavioral deficits induced by THC. Indeed, this alkaloid was able to reduce both neuro-inflammation and oxidative damage as manifested by the decreased IL-1β, MDA, and the antioxidant defenses as well, when co-administered with THC in the current study. These beneficial effects might be linked to the ability of Nic to activate α7nAChR subunit that represses NFκB (Yoshikawa et al. 2006). Such an effect activates the cholinergic anti-inflammatory pathway to lend a plausible explanation to Nic antioxidant and anti-inflammatory activities to hamper THC devastating effects on memory, euthymic mood, and decreased movement, as shown in the present work. In fact, Nic has been accounted for its therapeutic use in some inflammatory and oxidative stress-mediated diseases (Yoshikawa et al. 2006). Besides the antioxidant effect of Nic, as depicted herein and earlier (Kacham 2013), the stimulant nature of the alkaloid small doses (Rose et al. 2003) can lend credit to the increased locomotion in both single and Nic/THC20 groups.

THC did not alter serum AChE activity but suppressed that of BChE; both of which catalyze the hydrolysis of acetylcholine (Ach) (Chen et al. 2011). On the other hand, only Nic co-administered with THC10 normalized BChE. Though serum levels of both esterases are reflections of their brain contents, Abdel-Salam et al. (2016) reported that the hydrolysis of ACh was mainly carried out faster by the specific enzyme with a negligible role for the pseudo-enzyme after THC administration in naïve rats, indicating a minor role for BChE activation by Nic co-administration in memory consolidation.

Of note, decreased brain glucose is linked to impaired cerebral energy metabolism and memory deficit, while on the contrary, its elevation enhanced memory (Abdel-Salam et al. 2013). Previous data showed that the administration of THC had variable dose- and region-dependent effects related to the CB1R (Miederer et al. 2017) on the brain glucose content ranging from low to high compared to untreated animals. However, our investigation showed that the cannabis extract failed to alter the whole brain glucose content, whereas only the combined administration of THC/Nic was able to elevate the brain glucose content. Accordingly, this effect might afford a further explanation to the enhanced memory consolidation seen in THC/Nic rats suggesting an interaction between these addictive and recreational drugs.

The present data provides evidence for the facilitatory effects of Nic on memory and locomotion impaired by THC, besides euthymia via antioxidant and anti-inflammatory potentials. This was reflected by functional as well as neurochemical and neuronal enhancements when Nic was co-adjunct with THC to provide important insights for understanding the consequences of habitual cannabis and/or Nic consumption.

Compliance with ethical standards

The experiments were conducted in accordance with the ethical guidelines for care and use in handling laboratory animals and were approved by the Ethics Committee of the NRC (Permit Number 10069).

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdel-Salam OME, Salem NA, El-Shamarka MES, Ahmed NAS, Hussein JS, El-Khyat ZA (2013) Cannabis-induced impairment of learning and memory: effect of different nootropic drugs. EXCLI J 12:193–214

Abdel-Salam OME, Youness ER, Khadrawy YA, Sleem AA (2016) Acetylcholinesterase, butyrylcholinesterase and paraoxonase 1 activities in rats treated with cannabis, tramadol or both. Asian Pac J Trop Med 9(11):1089–1094

Bevan-Jones WR, Surendranathan A, Passamonti L, Rodriguez PV, Arnold R, Mak E, Su L, Coles JP, Fryer TD, Hong YT, Williams G, Aigbirhio F, Rowe JB, O'Brien JT (2017) Neuroimaging of inflammation in memory and related other disorders (NIMROD) study protocol: a deep phenotyping cohort study of the role of brain inflammation in dementia, depression and other neurological illnesses. BMJ 7(1):e013187

Bonsall DR, Kim H, Tocci C, Ndiaye A, Petronzio A, McKay-Corkum G, Molyneux PC, Scammell TE, Harrington ME (2015) Suppression of locomotor activity in female C57Bl/6J mice treated with interleukin-1β: investigating a method for the study of fatigue in laboratory animals. PLoS One 10(10):e0140678. https://doi.org/10.1371/journal.pone.0140678

Cabral GA (2001) Marijuana and cannabinoids: effects on infections, immunity, and AIDS. J Cannabis Ther 1(3/4):61–85

Chen X, Fang L, Liu J, Zhan CG (2011) Reaction pathway and free energy profile for butyrylcholinesterase-catalyzed hydrolysis of acetylcholine. J Phys Chem B 115(5):1315–1322

Chen W, Li Z, Guo Y, Zhou Y, Zhang Z, Zhang Y, Luo G, Yang X, Liao W, Li C, Chen L, Sheng P (2015) Wear particles promote reactive oxygen species-mediated inflammation via the nicotinamide adenine dinucleotide phosphate oxidase pathway in macrophages surrounding loosened implants. Cell Physiol Biochem 35:1857–1867

Degenhardt L, Hall W, Lynskey M (2002) Exploring the association between cannabis use and depression. Addiction 98:1493–1504

Ellman GL, Courtney KD, Valentino Andres JR, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2):88–90 IN1, 91–95

Filbey FM, McQueeny T, Kadamangudi S, Bice C, Ketcherside A (2015) Combined effects of marijuana and nicotine on memory performance and hippocampal volume. Behav Brain Res 293:46–53

File SE, Kenny PJ, Ouagazzal AM (1998) Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. Behav Neurosci 112:1423–1429

Fischedick JT, Glas R, Hazekamp A, Verpoorte R (2009) A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in *cannabis sativa*. L. Phytochem Anal 20(5):421–426

Freedland CS, Whitlow CT, Miller MD, Porrino LJ (2002) Dose-dependent effects of Δ^9-THC on rates of local cerebral glucose utilization in rat. Synapse 45(2):134–142

Gorun V, Proinov I, Baltescu V (1978) Modified Ellman procedure for assay of cholinesterases in crude enzymatic preparations. Anal Biochem 86:324–326

Gotti C, Clementi F (2004) Neuronal nicotinic receptors: from structure to pathology. ProgNeurobiol 74(6):363–396

Graziano A, Petrosini L, Bartoletti A (2003) Automatic recognition of explorative strategies in the Morris water maze. J Neurosci Methods 130(1):33–44

Guan ZZ, Yu WF, Nordberg A (2003) Dual effects of nicotine on oxidative stress and neuroprotection in PC12 cells. Neurochem Int 43: 243–249

Harris LJ, Ray SN (1935) Diagnosis of vitamin-C subnutrition by urine analysis. Lancet 1(71):462

Hu SS, Mackie K (2015) Distribution of the endocannabinoid system in the central nervous system. In: Pertwee RG (ed) Handbook of experimental pharmacology, vol 231. Springer, New York, pp 59–93

Iversen L (2003) Cannabis and the brain. Brain 126:1252–1270

Iversen L (2012) How cannabis works in the human brain. In: Castle D, Murray R, D'Souza DC (eds) Marijuana and madness. Cambridge University Press, Cambridge, pp 1–11

Jean-Gilles L, Braitch M, Latif ML, Aram J, Fahey AJ, Edwards LJ, Robins RA, Tanasescu R, Tighe PJ, Gran B, Showe LC, Alexander SP, Chapman V, Kendall DA, Constantinescu CS (2015) Effects of pro-inflammatory cytokines on cannabinoid CB_1 and CB_2 receptors in immune cells. Acta Physiol (Oxf) 214(1):63–74

Kacham R (2013) Role of nicotine in oxidative stress. Thesis submitted to the faculty of Missouri University of science and technology in partial fulfillment of the requirements for the degree master of science in chemistry. Masters Theses 5447

Kaushal N, Bansal M (2014) Cell signaling and gene regulation by oxidative stress. In: Oxidative stress mechanisms and their modulation. Springer India, pp 105–121

Knedel M, Bottger R (1967) Einekinetsche Methodezur Bestimmung der Aktivitat der Pseudocholinesterase. Klin Wochenschr 45:325

Kruk-Slomka M, Boguszewska-Czubara A, Slomka T, Budzynska B, Biala G (2016) Correlations between the memory-related behavior and the level of oxidative stress biomarkers in the mice brain, provoked by an acute administration of CB receptor ligands. Neural Plast 016, Article ID 9815092, 15 pages

López-Malo D, Sanchez-Martinez JJ, Romero FJ, Barcia JM, Villar VM (2016) Oxidative stress and the combined use of tetrahydrocannabinol and alcohol: is there a need for further research? React Oxyg Species 2(6):388–395

Madras BK (2015) Update of cannabis and its medical use. Report to the WHO Expert Committee on Drug Dependence. http://www.who.int/medicines/access/controlled-substances/6_2_cannabis_update.pdf?ua=1

Manzanares J, Julian MD, Carrascosa A (2006) Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. Curr Neuropharmacol 4(3): 239–257

Miederer I, Uebbing K, Röhrich J, Maus S, Bausbacher N, Krauter K, Weyer-Elberich V, Lutz B, Schreckenberger M, Urban R (2017) Effects of tetrahydrocannabinol on glucose uptake in the rat brain. Neuropharmacology 1(117):273–281

Nishikimi M, Roa NA, Yogi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46:849–854

Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr Rev 27(1):73–100

Patel SS, Mehta V, Changotra H, Udayabanu M (2016) Depression mediates impaired glucose tolerance and cognitive dysfunction: a neuromodulatory role of rosiglitazone. HormBehav 78:200–210. https://doi.org/10.1016/j.yhbeh.2015.11.010

Rose JE, Behm FM, Westman EC, Mathew RJ, London ED, Hawk TC, Turkington TG, Coleman RE (2003) PET studies of the influences of nicotine on neural systems in cigarette smokers. Am J Psychiatry 160:323–333

Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H (1994) Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroids 59: 383–388

Schoeler T, Bhattacharyya S (2013) The effect of cannabis use on memory function: an update. Subst Abus Rehabil 4:11–27

Sedeek M, Nasrallah R, Touyz RM, Hébert RL (2013) NADPH oxidases, reactive oxygen species, and the kidney: friend and foe. J Am Soc Nephrol 24(10):1512–1518

Simonin F, Valverde O, Smadja C, Slowe S, Kitchen I, Dierich A, Le Meur M, Roques BP, Maldonado R, Kieffer BL (1998) Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonistU-50,488H and attenuates morphine withdrawal. EMBO J 17:886–897

Slattery DA, Cryan JF (2012) Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc 7(6):1009–1014

Tai S, Fantegrossi WE (2014) Synthetic cannabinoids: pharmacology, behavioral effects, and abuse potential. Curr Addict Rep 1(2):129–136

Tanasawet S, Boonruamkaew P, Sukketsiri W, Chonpathompikunlert P (2017) Anxiolytic and free radical scavenging potential of Chinese celery (*Apium graveolens*) extract in mice. Asian Pac J Trop Biomed 7(1):20–26

The National Academies of Sciences, Engineering, and Medicine, Health and Medicine Division, Board on Population Health and Public Health Practice, Committee on the Health Effects of Marijuana: An Evidence Review and Research Agenda (2017) The health effects of cannabis and cannabinoids: the current state of evidence and recommendations for research. http://nationalacademies.org/hmd/Reports/2017/health-effects-of-cannabis-and-cannabinoids.aspx

Trinder P (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 6(1):24–27

Tullis LM, Dupont R, Frost-Pineda K, Gold MS (2003) Marijuana and tobacco: a major connection? J Addict Dis 22:51–62

Tunez I, Drucker-Colin R, Montilla P, Peña J, Jimena I, Medina FJ, Tasset I (2010) Protective effect of nicotine on oxidative and cell damage in rats with depression induced by olfactory bulbectomy. Eur J Pharmacol 627(1–3):115–118

Turner J, Mahlberg P (1984) Separation of acids and neutral cannabinoids in *Cannabis sativa* L. using HPLC. In: Agurell DW (ed) Chemical pharmacological therapeutic agents. Academic press, New York, pp 79–88

United Nations (2018) Global overview of drug demand and supply latest trends, cross-cutting issues. World Drug Report 2018 (United Nations publication, Sales No. E.18.XI.9)

Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R (2002) Behavioural and biochemical evidence for interactions between D9-tetrahydrocannabinol and nicotine. Br J Pharmacol 135:564–578

Viveros MP, Marco EM, File SE (2006) Nicotine and cannabinoids: parallels, contrasts and interactions. Neurosci Biobehav Rev 30(8):1161–1181

Volkow ND, Baler RD, Compton WM, Weiss SRB (2014) Adverse health effects of marijuana use. N Engl J Med 370(23):2219–2227

WHO (2018) Leading cause of death, illness and impoverishment (WHO global report on trends in tobacco smoking 2000–2025)

Wolff V, Schlagowski A-I, Rouyer O, Charles A-L, Singh F, Auger C, Schini-Kerth V, Marescaux C, Raul J-S, Zoll J, Geny B (2015) Tetrahydrocannabinol induces brain mitochondrial respiratory chain dysfunction and increases oxidative stress: a potential mechanism involved in cannabis-related stroke. Biomed Res Int 2015:1–7. https://doi.org/10.1155/2015/323706

Yoshikawa H, Kurokawa M, Ozaki N, Nara K, Atou K, Takada E, Kamochi H, Suzuki N (2006) Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-κB phosphorylation and nuclear factor-κB transcriptional activity through nicotinic acetylcholine receptor α7. Clin Exp Immunol 146(1):116–123