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Asian Pacific Journal of Tropical Medicine

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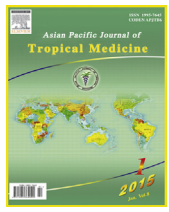

Original Research http://dx.doi.org/10.1016/j.apjtm.2016.09.009

# Acetylcholinesterase, butyrylcholinesterase and paraoxonase 1 activities in rats treated with cannabis, tramadol or both

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ARTICLE INFO

*Article history:*
Received 20 May 2016
Received in revised form 20 Aug 2016
Accepted 25 Aug 2016
Available online xxx

*Keywords:*
*Cannabis sativa*
Tramadol
Cholinesterases
Memory
Cognitive decline

ABSTRACT

**Objective:** To investigate the effect of *Cannabis sativa* resin and/or tramadol, two commonly drugs of abuse acetylcholinesterase and butyrylcholinesterase activities as a possible cholinergic biomarkers of neurotoxicity induced by these agents.

**Methods:** Rats were treated with cannabis resin (5, 10 or 20 mg/kg) (equivalent to the active constituent $\Delta^9$-tetrahydrocannabinol), tramadol (5, 10 and 20 mg/kg) or tramadol (10 mg/kg) combined with cannabis resin (5, 10 and 20 mg/kg) subcutaneously daily for 6 weeks. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were measured in brain and serum, respectively. We also measured the activity of paraoxonase-1 (PON1) in serum of rats treated with these agents.

**Results:** (i) AChE activity in brain increased after 10–20 mg/kg cannabis resin (by 16.3–36.5%). AChE activity in brain did not change after treatment with 5–20 mg/kg tramadol. The administration of both cannabis resin (5, 10 or 20 mg/kg) and tramadol (10 mg/kg) resulted in decreased brain AChE activity by 14.1%, 12.9% and 13.6%, respectively; (ii) BChE activity in serum was markedly and dose-dependently inhibited by cannabis resin (by 60.9–76.9%). BChE activity also decreased by 17.6–36.5% by 10–20 mg/kg tramadol and by 57.2–63.9% by the cannabis resin/tramadol combined treatment; (iii) Cannabis resin at doses of 20 mg/kg increased serum PON1 activity by 25.7%. In contrast, tramadol given at 5, 10 and 20 mg/kg resulted in a dose-dependent decrease in serum PON1 activity by 19%, 36.7%, and 46.1%, respectively. Meanwhile, treatment with cannabis resin plus tramadol resulted in 40.2%, 35.8%, 30.7% inhibition of PON1 activity compared to the saline group.

**Conclusions:** These data suggest that cannabis resin exerts different effects on AChE and BChE activities which could contribute to the memory problems and the decline in cognitive function in chronic users.

## 1. Introduction

*Cannabis sativa* L (family Cannabaceae) (*C. sativa*) has remained the most widely used and abused drug worldwide [1]. The two most common cannabis preparations are marijuana which is the dried flowing tops and leaves of the female plants and hashish which is the compressed resin. Cannabis has long been used through the history of mankind for its recreational properties. Cannabis consumers often report the subjective feeling of "being high", euphoria, altered time perception and increased sensual awareness. With long-term cannabis appear to impair several cognitive functions [2,3]. There is impairment of short-term and working memory that might persist for variable time after abstinence from cannabis [4,5]. There is also aggravation of pre-existing psychosis or even a likeness of developing psychosis in cannabis users [4]. Brain MRI scans indicate structural changes in

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Peer review under responsibility of Hainan Medical College.



Please cite this article in press as: Abdel-Salam OME, et al., Acetylcholinesterase, butyrylcholinesterase and paraoxonase 1 activities in rats treated with cannabis, tramadol or both, Asian Pacific Journal of Tropical Medicine (2016), http://dx.doi.org/10.1016/j.apjtm.2016.09.009

humans with a history of long-term [6] and heavy cannabis abuse while animal models shows neuronal degeneration [7,8] despite neuroprotection against excitotoxic brain injury (glutamate-induced death) being reported [9].

Only recently and with the identification of the cannabinoid receptor and their endogenous ligands, the biological action of cannabis beings to be delineated. The C21 terpenophenolic cannabinoids are the unique constituents of the *C. sativa* plant of which the principal psychoactive ingredient is $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC). Other cannabinoids such as cannabinol, cannabidiol, cannabivarin, cannabichromene, cannabigerol are devoid of psychotropic action and might even antagonize some of the pharmacological effects of $\Delta^9$-THC. This and other cannabinoids exist as their carboxylic acids and are converted (decarboxylated) into their corresponding phenols upon heating [10,11]. Cannabinoids or their endogenous ligands bind to cannabinoid receptors CB1 and CB2 with the former being predominantly expressed in the brain and spinal cord and thus mediates most of the effects of cannabis on the central nervous system. On the other hand, the CB2 receptor is mainly expressed on the surface of the immune cells in the periphery [12].

Tramadol is a frequently prescribed centrally acting analgesic with μ-opioid receptor agonist properties. It also inhibits the reuptake of serotonin, noradrenaline in the brain [13]. It is used to treat acute pain and of moderate to moderately severe chronic pain resulting from musculoskeletal disorders or that due to cancer [14]. The drug is becoming increasingly popular in several countries as a drug of misuse [15–17]. Subjects taking 675 mg or more of tramadol for 5 years or more exhibited an increase in comorbid anxiety, depressive, and obsessive-compulsive symptoms [17]. There is also evidence of memory impairing action for tramadol [18]. Cannabis users are more likely to report use of other illicit drugs [20] including tramadol [19].

In brain, central cholinergic neurotransmission is crucial for cognitive functions including learning and memory formation [21]. Inhibitors of brain acetylcholinesterase such as donepezil and rivastigmine are the drugs being used to treat the cognitive decline due to aging or Alzheimer's disease by increasing extracellular acetylcholine, the signaling neurotransmitter of the cholinergic system [22]. Changes in central cholinergic activity thus will have an important impact on cognitive functions [21]. The aim of this study was therefore to investigate the effect of cannabis and/or tramadol on the activities of brain acetylcholinesterase and plasma butyrylcholinesterase, the enzymes involved in the hydrolysis of the acetylcholine [23]. We in addition measured the activity of paraoxonase-1 (PON1) in serum of rats treated with cannabis and/or tramadol. The PON1 enzyme is involved in the detoxification of several organophosphorus compounds and many other xenobiotics and changes in its activity have been associated with a number of neurologic disorders [24,25].

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats, obtained from Animal House of the National Research Centre, Cairo, weighing between 130 and 140 g were group-housed under temperature- and light-controlled conditions with standard laboratory rodent chow and water provided *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

### 2.2. Drugs and chemicals

*C. sativa* resin (Hashish) and tramadol were kindly provided by the Laboratory of Forensic Sciences of Ministry of Justice (Cairo, Egypt). Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A).

### 2.3. Preparation of cannabis resin extract

Cannabis resin extract was prepared from the dried resin of *C. sativa*. The extraction was performed using chloroform according to the method of Turner and Mahlberg [26] with modification. In brief, 10 g of the resin was grounded in a mortar, subjected to oven heat (100 °C) for 1 h to decarboxylate all its cannabinolic acids content. The resin was extracted in chloroform overnight and filtered. The filtrate was evaporated under a gentle stream of nitrogen and stored at 4 °C and protected from light in an aluminum-covered container. 1 g of the residue (dry extract) was suspended in 2% ethanol-saline. $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) content was quantified using gas chromatography–mass spectrometry (GC–MS). The resin contained ~20% $\Delta^9$-THC and 3% cannabidiol.

### 2.4. Study design

Rats were treated with *C. sativa* resin extract at 5, 10 or 20 mg/kg (expressed as $\Delta^9$-tetrahydrocannabinol), tramadol at 5, 10 or 20 mg/kg or tramadol (10 mg/kg) in combination with *C. sativa* resin (5, 10 or 20 mg/kg) subcutaneously daily for 6 weeks. Rats were randomly divided into ten groups, six rats each. Group 1 received the vehicle (0.2 mL saline) daily. Group 2, 3, 4 received *C. sativa* resin at the doses of 5, 10 and 20 mg/kg, subcutaneously daily. Groups 5, 6, 7 received tramadol at doses of 5, 10 and 20 mg/kg subcutaneously daily. Groups 8, 9, 10 received tramadol at 10 mg/kg in combination with *C. sativa* resin (5, 10 or 20 mg/kg, subcutaneously daily). Rats were then euthanized by decapitation under ether anesthesia for tissue collection. The brain of each rat was rapidly dissected and snap-frozen in liquid nitrogen. 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 9 000 g for 5 min at 4 °C. The supernatant was stored at −80 °C until further analysis.

### 2.5. Determination of acetylcholinesterase activity

The procedure used was a modification of the method of Ellman *et al.* [27] as described by Gorun *et al.* [28]. The principle of the method is the measurement of the thiocholine produced as acetylthiocholine is hydrolyzed. The color was read immediately at 412 nm.

### 2.6. Determination of butyrylcholinesterase activity

Butyrylcholinesterase activity was measured spectrophotometrically using commercially available kit (Ben Biochemical



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