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Molecular and histological changes in cerebral cortex and lung tissues under the effect of tramadol treatment

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ABSTRACT

Tramadol abuse is one of the most frequent health problems in Egypt and worldwide. In most cases, tramadol abused by men face a problem with premature ejaculation. Tramadol like other opioids induces a decrease in plasma antioxidant levels, which may reflect a failure of the antioxidant defense mechanism against oxidative damage. The present work aimed to study the possible deleterious effects of oral administration of tramadol on brain and lung tissues in rats. Twenty adult male albino rats were divided into two groups; a control administered with normal saline and tramadol-treated (40 mg/kg b.w.) group for 20 successive days. At the end of experimental period, blood was collected and specimens from brains and lungs were taken for histopathological and molecular studies. Malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities were measured in serum of control and tramadol-treated groups. Brain and lung specimens were histopathological evaluated using light microscopy. The expression levels of apoptotic related genes; Bcl-2, Bax and Caspase-3 were study in brain and lung tissues using RT-PCR analysis. We recorded a significant increase MDA level, while antioxidant enzymes; GSH, SOD and CAT were significantly decreased after tramadol-treatment. The obtained results revealed that tramadol induced a remarkable histomorphological changes in rats' brains (cerebral cortex and hippocampus) and severe histopathological changes in rats' lung when compared to that of control. On molecular level, the expression of the pro-apoptotic Bax and Caspase-3 showed a significant increase whereas the anti-apoptotic Bcl-2 decreased markedly indicating that tramadol is harmful at cellular level and can induce apoptotic changes in brain tissues. Our data confirmed the risk of increased oxidative stress, neuronal and pulmonary damage due to tramadol abuse. Although tramadol is reported to be effective in pain management, its toxicity should be kept in mind.

1. Introduction

Tramadol belonged to a synthetic opioid of the aminocyclohexanol group; it is analgesic used effectively in pain treatment in experimental and clinical studies [30]. Tramadol administration efficiently alleviates pain in acute and chronic conditions [55]. The neurotoxicity of abusing drugs are usually related to oxidative stress, mitochondrial dysfunction, apoptosis, and inhibition of neurogenesis [11]. Tramadol toxicity and abuse were announced as an atypical opioid as it produces analgesia by the synergism between the parent drug and its o-desmethylated metabolites [65]. It follows a complex pathway that is responsible for opioid receptor-mediated analgesia and inhibition of neuronal re-uptake of nor-epinephrine and serotonin [44,59].

At a cellular level, lipid peroxidation (LPO) production reflects the degree of toxicity of opioids [32], because a significant elevation in LPO was found in rats receiving an acute cocaine dose [33]. Similarly, LPO was elevated significantly among chronic heroin dipsomaniac [41]. The previous study found that glutathione level in rat hepatocytes was marked decrease after incubated with morphine, which causes hepatocytes death [68]. Furthermore, the levels of serum Malondialdehyde (MDA) was elevated after treatments with morphine and tramadol indicating an increase in LPO production [3].

Oxidative stress refers to the loss of balance between Reactive Oxygen Species (ROS) level and antioxidant defense system. [8] reported that ROS could induce apoptosis. The free-radicals overproduction causes a mortality to immature cultured cortical neurons [43] and directly produce a damage to DNA [60]. Among

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body organs in the body, the central nervous system (CNS) suffers from oxidative abuse more than other organs. This effects related to plenty of redox-active transition metal ions found, and the relative death of antioxidant defense system [51].

Brains can be a target to the free radicals, besides the high oxygen consumption and high polyunsaturated fatty acids (PUFA) levels present, that makes nerve tissues even suffer more from oxidative stress [18]. There is a direct involvement of the cellular redox status in the initiation, activation and the functioning of the apoptotic machinery as reported by Oikawa et al. [38]. Scaiano et al. [54] found that free radicals inducing damage at the cellular level causing aberrations in chromosomes.

Apoptosis performs an important role in embryogenesis, normal development, and adult tissues sustaining [53,25]. Cell death, through the apoptotic pathway, is regulated by many genes expression and/or proteins activation [62]. Apoptosis initiation and execution depends on the activation of receptor and/or mitochondrial-dependent death pathways [2,10,22]. Bax (Bcl-2 associated-X-protein) is a gene that first discovered among pro-apoptotic members of a family of proteins known as Bcl-2 (B-cell lymphoma-2) [39]. In CNS, Bcl-2 suppresses apoptosis against different stimuli that cause neuronal death [1,6]. Indeed, targeting of Bcl-2 family proteins to mitochondria has been demonstrated to regulate death induced by a variety of stimuli via apoptosis [39,28]. In addition, Caspases (cysteine-aspartic proteases) are a family of proteins belonged to cysteine proteases, that play a role in apoptosis [10]. Stefanis et al. [61] reported that Caspase-3 activation might play a role in apoptosis initiation in nerve cells.

The respiratory system is invariably exposed to the drugs. Drugs affect it either directly or secondarily on the temporary or permanent basis. Illicit drugs and psychoactive substances affect all anatomical lung compartments producing diverse morphological changes. Three classes of drugs can produce a respiratory manifestations; opiates, stimulants and cannabinoids [20]. Pulmonary patho-histological findings are include; edema, pulmonary hemorrhage and appear of siderophages, pulmonary artery medial hypertrophy, panacinar emphysema, bronchiolitis obliterans, interstitial pneumonia or fibrosis [24]. In lung and endometrial tissues, Bcl-2 protein localized mainly in mitochondrial membrane and played a main role to protect tissues from apoptosis [45,47,48]. Increased in Bax cytoplasmic expression in lung cancer cell enhances apoptosis [46,49].

Tramadol is used in many countries by addicts orally every day out-of-law and away from doctor prescriptions. Therefore, we care about studying the effect of Tramadol injection by mouth, to evaluate its toxicity on body organs of normal rat's model. The objective of this study is to investigate histopathological changes and the alteration in the apoptotic genes expression in both brain and lung tissues and to estimate the biochemical changes induced in serum by tramadol treatment.

2. Materials and methods

2.1. Chemicals

Tramadol tablets were obtained from October Pharma Co. (Giza, Egypt). Each tablet contains 225 mg tramadol hydrochloride. Kits for; lipid peroxidation, reduced glutathione, superoxide dismutase and catalase; were purchased from Biodiagonistic Co. All other chemicals were of the highest quality available.

2.2. Animals and treatments

Twenty adult male albino rats (4 months old) weighing (120–140 g) obtained from the Animal House, The Egyptian Co. for Vaccines Production at Helwan, were used in our study. Rats were rearing and kept at room with controlled light at photoperiods of 12 h dark and 12 h light and a temperature around $28 \pm 2\,^{\circ}C$. Aswan University approved the experimental design through the Animal Care and Use Committee.

The rats were divided into two groups (control and tramadol groups), 10 animals each. Control (C) group was treated daily by normal saline (0.9% NaCl, oral) for 20 days. Rats, in tramadol (T) group, were given tramadol hydrochloride orally for 20 days at a dose equivalent to 40 mg/kg body weight [4,15]. Rats were sacrificed by decapitation 24-h after last tramadol dose injection.

2.3. Biochemical studies

Blood were collected in non-coated serum separating tubes. The blood was centrifuged at 3000 rpm for 15 mins to separate serum. Sera were used for measurement of MDA, GSH, SOD, and CAT activities in both control and tramadol-treated groups.

2.4. Determination of lipid peroxidation and antioxidant enzymes activities

LPO was estimated in the serum and was expressed in terms of MDA content [37]. CAT activity was analysis by the method of Sinha [58], SOD activity was assayed according to Misra and Fridovich [35], and GSH was measured with the method published by Carlberg and Mannervik [7].

2.5. Histopathological examination

After scarification, specimens from the brain and lung were taken from the two studied groups. Tissues were fixed in 10% neutral buffer formalin, embedded in paraffin, sectioned at 5 μm and prepared for the following stains:

1. Harris's Hematoxylin and Eosin (H&E) stain [19].
2. Toluidine blue stain [19] for Nissl granules.

2.6. Isolation of total RNA (tRNA) from brain and lung tissues

Frozen brain and lung samples were thawed and used for RNA extraction. Homogenization of 200 mg of frozen tissue samples was carried in 1 mL TriZol (Invitrogen, Carlsbad, CA) using a Polytron 300D homogenizer (Brinkman Instruments, Westbury, NY). tRNA extraction was done by Trizol reagent following the method of [9]. The tRNA obtained was free from contamination with DNA and protein. Nucleic acid concentration was assayed by optical density (OD) at 260 nm wavelength (Smart-Spec; Bio-Rad Laboratories, Hercules, CA). tRNA integrity was estimated using an Agilent bioanalyzer (model 2100; Agilent Technologies, Foster City, CA). The purity and integrity of tRNA was evaluated by dividing the OD at 260 nm over OD at 280 nm. The high purity tRNA samples (OD 260/280 >1.8) were used to run the further manipulation.

2.7. Reverse transcription-polymerized chain reaction (RT-PCR) analysis

The reverse transcription (RT) steps were carried out using RT-enzyme kit. Each 20 μL reaction mixture contained 5 μL Oligo (dT) (10 μM), 1 μL dNTP (10 μM), 4 μL First Strand Buffer (5X), 1 μL DTT (0.1 M), 0.2 μL super Script III Reverse Transcriptase (200 U/μL), different quantity of tRNA template (according to tRNA concentration) and RNase-free water to make up the total volume (20 μL). Thermal cycling conditions to prepare the complementary-DNA (cDNA) consisted of; 25 °C for 5 min, 50 °C for 45 min and 70 °C for 15 min and finally kept at 4 °C for 5 min.