A HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF ADULT RATS’ BRAIN AFTER LONG-TERM EXPOSURE TO AMADOL (TRAMADOL HYDROCHLORIDE)

By

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ABSTRACT

Tramadol hydrochloride (TH) is an atypical opioid synthetic agent which is a centrally acting analgesic, used for treating moderate to severe pain with fewer side effects than traditional opioid medications. The present work aimed to study the dose-dependent possible deleterious effect of long-term administration of TH as well as a 4-weeks spontaneous recovery period to evaluate the reversibility of the toxic effects on the brain tissues of the rats. Forty adult male albino rats were divided into 4 groups. Group I (control), group II (low dose) was treated orally with TH (30 mg/kg/day) and group III (high dose) was treated orally with TH (60 mg/kg/day) for 4 weeks. Group IV (follow up) was treated as group III and then held for 4-weeks recovery period. At the end of experimental period, rats were sacrificed, histopathological and immunohistological (IHC) examinations of the brains were carried out. The obtained results revealed that both low and high doses of TH produced remarkable histomorphological changes in rats’ brains (cerebral cortex {CC} and hippocampus {HC}) as compared to control and were more pronounced in high than low dose group and in both doses when compared to that of control. On the other hand, group IV showed remarkable regression of the total degenerative changes induced by TH with some residual effect. This was noted by nearly normal morphology of brain tissues and marked significant decrease of p53 and Bax along with significant increase of Bcl-2 protein expressions when compared to those of group II and III treated rates.

INTRODUCTION

Tramadol hydrochloride (TH), a synthetic opioid of the aminocyclohexanol group, is a centrally acting analgesic that has been proved to be effective in both experimental and clinical pain treatment without causing serious cardiovascular or respiratory side effects (Lehmann, 1997). Unlike traditional opioid receptor agonists, TH has limited effects on respiratory or cardiovascular parameters as well as low abuse or dependence potentiality. Oral and parenteral TH effectively relieve acute or chronic; moderate to severe pain conditions (Scott and Perry, 2000). Its prolonged use in chronic cancer and non-cancer pain (e.g. low back, osteoarthritis,
neuropathic, fibromyalgia, migraine ..etc) is well established (Babul et al., 2004). Also, it has been suggested that TH could be effective for alleviating symptoms of depression, anxiety, and phobias (Rojas-Corrales et al., 2004). Additionally, TH seems to have a specific role in the treatment of opiate withdrawal (Threlkeld et al., 2006) and premature ejaculation (Salem et al., 2008).

The most frequent adverse effects of TH include constipation, nausea, dizziness, headache, somnolence and vomiting (Sittik et al., 2006). The most serious adverse reactions include confusion, hallucinations, convulsions, serotonin syndrome, and hypersensitivity reactions. Also, several reports of withdrawal symptoms with long-term TH usage were reported (ADRAC, 2003).

A better understanding of the fundamental mechanisms involved in apoptosis has made it possible to define its real significance in many areas of cell biology and, more recently, in pharmacology and toxicology (Feldmann, 2006). Apoptosis provides a mechanism for the disposal of cells damaged by toxicants without perturbing the homeostatic balance of their environment. However, the importance of apoptosis in toxicology has been underestimated given the difficulty of identifying apoptotic cells either in vivo or in vitro models (Gómez-Lechón et al., 2008).

The molecular mechanisms of opioid-induced apoptosis have not been established yet. Various key proteins are involved in the regulation of programmed cell death (Sastry and Rao, 2000). Some members of the Bcl family, such as Bcl-2 and Bcl-xL are known anti-apoptotic proteins that suppress apoptosis. Whereas, the cytoplasmic expression of Bax is pro-apoptotic that enhance apoptosis. Bcl-2 protein localized mainly in the mitochondrial membrane has been shown to play an important role in protecting tissues from apoptotic cell death (Yuan and Yankner, 2000).

The p53 tumor suppressor protein also plays a central role in cell cycle arrest and apoptosis (Polyak et al., 1997). p53 is a pro-apoptotic short-lived protein (its half life is approximately 10-30 min) and constitutively expressed at low levels in most cell types including neurons (Soussi, 2000). Normally, several negative regulatory mechanisms that control p53 function were reported. Cytoplasmic p53 directly binds to Bcl-xL forming complex (Chipuk et al., 2005). Also, within the nucleus, human homolog double minute-2 / murine double minute-2 (HDM2/MDM2) binds p53 and mediates its transport into the cytosol, where it is ubiquitinated and then degraded by the proteasome (Prives and Hall, 1999).

Bax has been shown to contain p53-
binding sites in promoter and regulated in response to p53 in a number of systems (Miyashita and Reed, 1995). Under normal conditions, Bax is present in monomeric form in the cytosol or loosely attached to membranes (Wolter et al., 1997). Unlike Bax, Bcl-2 is mainly localized as an integral mitochondrial membrane protein, and forms heterodimers with Bax to prevent mitochondrial changes in apoptosis (Hockenbery et al., 1990). Hence, dysregulation between pro- and anti-apoptotic proteins may initiate or inhibit the development of cellular apoptosis. Unregulated excessive apoptosis may be the cause of various degenerative and autoimmune diseases that are characterized by an excessive loss of normal or protective cells (Giordano et al., 1997).

The objective of the present study was to examine the dose dependent possible deleterious effect as well as a 4-weeks spontaneous recovery period to evaluate the reversibility of the toxic effects of long-term administration of TH on the brain histopathology architectures in normal adult male albino rats. On the basis that p53, Bax, and Bcl-2 proteins are vital regulators of apoptosis, it is of consequence to find out whether the expressions of these proteins are involved in the mechanisms of TH induced apoptosis by determining the immunohistochemical (IHC) expression of p53, Bax, and Bcl-2 in rats’ brain.

**MATERIAL AND METHODS**

**Drugs:** Commercially available Amadol capsules. Each capsule contains 50 mg Tramadol hydrochloride [TH] (Amadol, manufactured by ADWIA Co. S.A.E., 10th of Ramadan City, Egypt).

**Animals and Experimental design:**
Forty adult male albino rats, weighing 160-180 g, were obtained from Helwan Farm For Experimental Animals and kept under standard conditions throughout the experimental work. They were maintained on a standard diet with free access to water. After acclimatization for 2-weeks, they were randomly assigned into 4 groups of ten rats each as follows:

- **Group I (Control):** Each animal received 1ml normal saline 0.9% orally by gavage for 4 weeks.
- **Group II (Low Dose):** Each animal received 30 mg/kg/day (1/10th LD$_{50}$) of TH orally by gavage for 4 weeks (Matthiesen et al., 1998).
- **Group III (High Dose):** Each animal received 60 mg/kg/day (1/5th LD$_{50}$) of TH orally by gavage for 4 weeks.
- **Group IV (Follow-up):** Animals received the same dose as group III then held for 4 weeks nondosing spontaneous recovery period after withdrawal of the drug.
All doses of TH were delivered in a volume of 1 ml normal saline. At the end of each experimental period and under ether anesthesia all animals were sacrificed after 24 hours of the last dose. Craniotomy was performed and intact brains were dissected and removed for histopathological and immunohistochemical studies.

**Histopathological and Immunohistochemical Studies:**

The brains were removed and dissected into several regions (cerebral cortex and hippocampus) according to the guidelines of Glowinski and Iversen (1966). The excised brain regions were isolated and washed with normal saline followed by 50 ml (4%) paraformaldehyde of in phosphate-buffered saline (PBS), postfixed in 10% formalin for 7 days, and then paraffin embedded. Paraffin blocks were sectioned into 4-5 µm thick sections.

Serial sections of brain were stained with haematoxylin and eosin (H and E) according to Drury and Wallington (1980) to be examined by light microscopy for histopathological changes.

For immunohistochemical (IHC) study, brain sections were placed on poly-l-lysine coated clean slides and stained with anti-p53, anti-Bcl-2, or anti-Bax according to the method of Palermo et al., (2004).

Scoring of IHC stained neurocytes was done under light microscopy and away from lesions. Brown immunostaining of nuclei (for p53), cytoplasm (for Bax), and perinuclear membrane (for Bcl-2) were considered as positive apoptotic neuronal cells. Semi-quantification analysis of the apoptotic index (AI) was determined by counting a total of at least 1000 cells per slide subdivided in 10 fields chosen randomly at x400 magnification. AI% = [number of positive cells/total number of calculated cells] x 100 is the percentage of positive cells in 1000 cells (Xu et al., 2007).

**Statistical Analysis**

One-way analysis of variance (ANOVA) was performed by using the SPSS 16 for Windows software system (SPSS Inc, Chicago, IL) was used for data analysis. Statistical significance was taken at p<0.05 When the difference between treatment groups was significant, post hoc analysis was carried out by applying the LSD. All data were presented as mean ± SD.

**RESULTS**

No deaths were observed in any of the control or treated groups during the 4 weeks treatment as well as during the 4 weeks recovery period.

**Histopathological study of brain (Plates 1-10):**

Light microscopic examination of
The previously mentioned degenerative changes were markedly intensified in different brain regions of group III treated rats. Morphological examination revealed marked cortical layers disorganization, vacuolated foci with cellular loss, intense eosinophilic staining of neuropil, non-specific inflammatory cells infiltration, and increased number of apoptotic and red neurons. Additionally, HC neuronal cells showed less histopathological changes than those of CC. Shrunken apoptotic cells as well as degenerated and necrotic cells, and red neurons with shifting of nucleus towards axon were detected. These lesions were more marked with high dose TH-treated rats.

However, light microscopic examination of brain regions of group IV (follow up), after 4 weeks recovery period, showed return of brain tissues towards normal morphology as evidenced by remarkable regression of the total degenerative changes that induced by TH, but still slightly different when compared with control.

**Immunohistochemical study of brain (Table 1, Plates 11-34):**

The AI of CC and HC cells were shown in table 1. In the control group, only a small number of immunostained positive cells were detected. Whereas, evaluation of high dose TH group (group III) showed significantly higher values in comparison to control, low dose TH (group II) and

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*Khodeary et al...*
DISCUSSION

In the current study, the effect of long-term TH on the brain morphology by histopathological and immunohistochemical investigations were studied in rats.

Concerning the present work, both low dose and high dose groups of TH treated rats showed neurohistopathological alterations in their brains. However, marked cytopathological injury reported in group III than group II displayed dose-dependent effect of TH on studied brain regions. Whereas, rats examined after the recovery period unlikely showed complete recovery (did not return back to normal control) but marked reduction in cellular damage was observed when compared to TH-treated groups II and III. In general, there was appreciable improvement in the follow up group and longer time may be required by neurocytes for complete recovery. In rats, TH preferentially gains access to the brain tissues compared to its active metabolite (Tao et al., 2002). It was postulated that TH potentially induced neurotoxicity in rabbits by decreasing membrane fluidity of the blood brain barrier secondary to loss of unsaturation and serious changes in the structural concentrations and numbers of fatty acids as reported by Alici et al. (2003).

Evidence of acute eosinophilic neuronal degeneration by light microscopy was
seen in rats receiving large-dose of different μ-opioid receptor agonist such as fentanyl and its congener. The degenerating red neurons were characterized by retracted brightly eosinophilic cytoplasm, nuclear pyknosis, pale and/or vacuolated neuropil "malacia" surrounding the eosinophilic neurons. The authors suggested that there may be a role for either cholinergic or catecholaminergic neurotransmitters in the genesis of opioid-induced brain damage (Kofke et al., 1996a). Furthermore, experimental administration of alfentanil, another μ-opioid receptor agonist, in large doses, produced histopathologic alterations in rats’ HC with more affection of the large pyramidal neurons than the granular neurons of the dentate gyrus (Kofke et al., 1996b).

Chronic opiate treatment induced significant alterations in the ultrastructure morphology of the rats’ neurons as evidenced by indentation of nuclei, fragmentation and degranulation of rough endoplasmic reticulum, as well as the disaggregation of polyribosomes and myelinoid body deposition. The investigators hypothesized that these changes might reflect a defect or inhibition in protein synthesis (Miao et al., 1997). The appearance of excessive myelinoid bodies after chronic opiate treatment in rats is an indicative of severe damage to mitochondria which carry out most of cellular oxidation and supply cells with energy, and thus may lead to energy failure in the neurons (Alberts et al., 1994).

Hauser et al. (1996) and Eisch et al. (2000) concluded that chronic opiate exposure can decrease the proliferation and survival of new neurons in the mature adult brain by acting directly on the neurocytes progenitor population so decrease their proliferation and DNA synthesis via an opioid action at the μ-opioid receptor.

Tramadol has the potential to trigger two dramatic events namely seizures (due to lowering of the seizure threshold) and serotonin syndrome (due to increase of cerebral serotonin activity). These may develop during monotherapy either at routine or dose-dependent manner (Vizcaychipi et al., 2007).

Houlihan (2004) stated that prolonged therapy with TH induced rise in central serotonin level by synergistic enhancement of serotonin release combined with inhibition of its reuptake. Also, Ogata et al. (2004) suggested that the action of TH on serotonin receptors is mediated through competitive displacement of the serotonin binding to the receptors, rather than via activation of the protein kinase C pathway. Furthermore, TH produced down regulation of rat brain α-2-adrenergic receptors which in turn inhibit serotonin reuptake (Faron-Górecka et al., 2004).
Additionally, attenuation of sympathetic tone by ganglionic blockade has been shown to have protective effects in brain ischemia. Presumably, this blockade decreases autonomic input into the brain from extracranial sources with subsequent interference with neurotoxic effects of catecholamines. Furthermore, preischemic depletion of catecholamines with reserpine induced neuroprotective effect through abolishing direct catecholamines neurotoxicity (Busto et al., 1985; Werner et al., 1990).

Degenerated neuronal cells seem to be exhausted by the sustained augmented activity in response to continuous administration of TH. It was shown that rats exposed to stress factors, including chemical substance, developed disturbance in the function of serotonin receptors in nerve cells and other tissues with subsequent occurrence of uncontrollable cholinergic action causing vasoconstriction and ischemia (Sakurai-Yamashita et al., 2003). Moreover, stress-induced modulation of dopamine D1 and serotonin receptors functions through hyperactivation of cyclic adenosine 3-5- monophosphate which triggered neuronal degeneration as clarified by Tsukada et al. (2004).

An immune-mediated mechanism may be involved in TH-induced neuronal cell injury. Cotran et al. (1994) announced that whenever a cellular degeneration is setting in, there is a cellular concomitant release of interleukins and chemotactic cytokines from these cells, which cause local vasodilatation and attraction of non inflammatory cells that subsequently stimulate proliferation and activation of neuroglial cells by release of fibroblast growth and tumor necrosis factors.

In the current study, despite the remarkable regression of the neuropathological changes in group IV, after 4 weeks recovery period, morphological pattern of brain regions still showed some residual toxic effect of TH. Not only inhalation but also intravenous consumption of heroin can lead to toxic leukoencephalopathy. Despite initially severe neurological symptoms, a gradual improvement of neurological symptoms and signs was observed after 4 weeks of heroin exposure and nearly complete recovery was noticed after 6 months of exposure (Maschke et al., 1999). Likewise, Gottfried et al. (1997) described a case of oral morphine toxicity in which recovery of neurological function was significant but incomplete after 6 months of exposure.

On the other hand, in a neurotoxicity study, TH was administered orally to rats using dosages up to 40 mg/kg/day. Both histopathological and electron microscopy evaluations of different brain regions recognized no treatment-related morphological lesions (Matthiesen et al., 1998). These
findings disagreed completely with the present study.

The current study demonstrated that statistical analysis of AI was significantly different in TH-treated groups and follow-up group as compared to control group with the following ranking order: group III > group II > group IV. Whereas, group IV displayed significant regression of apoptotic changes (previously induced by TH) towards normal value when compared with group II and III treated rats (but still significantly different from control). The brain pro-apoptotic p53 and Bax protein expressions were significantly increased conversely to the anti-apoptotic Bcl-2 protein expression, which depicted significant decrease in the TH treated groups as compared to control as well as follow up groups. Thus, p53 expression was positively related to pro-apoptotic Bax protein expression, and negatively to Bcl-2 protein expression. The up-regulation of the p53 and Bax besides down-regulation of Bcl-2 in rat neurocytes were significantly pronounced in TH treated group with high dose than low dose indicating dose dependent effects of TH. However, marked significant regression of these changes towards normal expressions (but did not return completely to normal) was noticed in follow-up group indicating appreciable improvement of brain functions during the spontaneous recovery period. These data also suggested that activation of apoptotic cell death pathway namely overexpression of p53 and Bax as well as suppression of Bcl-2 proteins may play an important role in TH-induced brain damage.

Opioids may be involved not only in neuronal survival but also in programmed cell death. There are conflicting results in the literature concerning the effects of opioids on apoptosis. In vitro studies using specific cell lines showed that opioids might induce or enhance apoptosis (Singhal et al., 1998).

In a study carried out by Atici et al. (2005), red neurons were found in TH and morphine groups but not in the control group. The total number of red neurons was not different between treated groups. Thus, chronic use of TH in increasing doses was found to cause red neuron degeneration in the rats’ brains. The authors speculated that promoted synthesis of Bax might have contributed to increased neuronal apoptosis due to chronic use of TH.

Buprenorphine, a semi-synthetic analgesic opioid derivative with partial agonist effect at the µ-opioid receptor and closely similar in structure to TH, was shown to induce very rapid apoptosis in rodent neuroblastoma and glioma cell lines (Kugawa et al., 2000). This fast apoptosis may be mediated by the p53/Bax pathway (Miyashita and Reed, 1995).
Furthermore, morphine has been shown to increase the production and release of nitric oxide and has been shown to induce apoptosis in macrophage populations by a p53-dependent mechanism that is naloxone sensitive (Singhal et al., 1998). These immune cells, in general, possess µ-opioid receptors that are comparable to those in neurons (Sharp et al., 1998). Also, prolonged morphine administration resulted in an up-regulation of the pro-apoptotic Bax protein besides a down-regulation of the anti-apoptotic Bcl-2 protein (Mao et al., 2002). This intracellular imbalance in Bax/Bcl-2 ratio accelerated apoptosis of lymphocytes by morphine with subsequent activation of caspase 3 (Xu et al., 2004).

In many types of neurons, activation of p53 apoptotic pathway may be mediated by a wide range of insulting agents such as DNA damage, ischemia/hypoxia, hypoglycemia, and excitotoxicity to oxidative stress (Morrison et al., 2003). This up-regulation of p53 in multiple neuronal populations suggests that p53 is a key factor involved in neuronal death in response to different forms of acute insults and chronic neurodegenerative conditions (Culmsee and Mattson, 2005).

Another possible mechanism of TH-induced brain damage is the decrement in the rat brain activities of Na\(^{+}/K\(^{-}\), Mg\(^{2+}\) and Ca\(^{2+}\)-dependent ATPases with subsequent decrease in ATP turnover and energy metabolism as well as loss of mitochondrial membrane transport functions (Cheetan et al., 2007). In addition, TH and/or its active metabolite may produce excessive release of ROS leading to single- or double-strand DNA breaks (Klaunig and Kammendulis, 2004).

The accumulation of DNA strand breaks is a well-established stimulus for p53 activation. In addition, oxidative damage of proteins involved in cell cycle regulation or DNA repair may contribute to accumulating DNA damage and finally to activation of p53 which, in turn, mediates either DNA repair or apoptosis (Culmsee and Mattson, 2005). The molecular mechanisms by which p53 is activated and accumulates under these conditions of cellular stress may include phosphorylation or acetylation of p53, interference with the HDM2/MDM2 system, and proteasome inhibition (Brooks and Gu, 2003). Such modifications either increase the stability of p53 or directly enhance its DNA-binding affinity (Culmsee and Mattson, 2005). p53 can cause apoptosis by inducing the expression of Bax that cause mitochondrial membrane permeabilization (Jeffers et al., 2003). After activation, Bax translocates to mitochondria and becomes an integral membrane protein and cross-linkable as a homodimer. When inserted into membranes, Bax may form channels allowing for the release of apoptosis-
related proteins, such as cytochrome c, from the mitochondria to propagate the apoptotic pathway (Wolter et al., 1997). p53 may also promote apoptosis through transcriptional repression of survival factors such as Bcl-2. p53 binds to and interferes with transcriptional activities of certain cofactors such as p300 or cyclic adenosine monophosphate-response element-binding protein. Blocking of these limited cofactors may be sufficient to block the activity of other transcription factors that are required for constitutive survival signaling in neurons (Wadgaonkar et al., 1999).

Repetitive or chronic exposure to uncontrollable stressor agents including drugs will gradually initiate a cascade of processes in brain which eventually leads to profound alterations in the electrical characteristics, morphology, suppression of proliferative capacity or neurogenesis and induction of neurocytes apoptosis of brain cells. However, animals subjected to a spontaneous recovery period of rest after chronic stress showed remarkable reversibility of these effects (Joëls et al., 2007). In the HC, chronic stress seems to reduce apoptosis, while in the CC increased apoptosis has been observed. Interestingly, IHC staining of HC and CC neurocytes of chronically stressed animals showed alterations of cellular apoptosis machinery. However, after 3-weeks recovery period, most of these alterations were normalized, indicating reversibility of stress related neurocytes apoptosis (Heine et al., 2004).

Withdrawal of the stressor neurotoxic agent, TH, was associated with appreciable reverse of the IHC staining pattern of the tested proteins towards normal activities, namely, down-regulation of p53 and Bax as well as up-regulation of Bcl-2 in group IV after 4 weeks recovery period. Although a marked but not complete recovery was observed in the current study, it is largely accepted that the ongoing process of neurocytes repairs requires a longer time period to achieve complete restoration to normal brain morphology. The nervous system differs from many other body organs by its low regeneration capacity (Kyrklund, 1992). The neurotoxicity is generally at least partially reversible, but complete recovery may take many months (Mollman, 1990). In contrast, red neuron degeneration was found even seven days after the last dose of fentanyl, this neurotoxicity effect was thought to be permanent rather than a transient effect (Kofke et al., 1996a). Hence, the duration of the recovery period should be taken into consideration during the analysis of the neuro-regenerative capacity after TH withdrawal.

In summary, administration of TH to rats induced dose-dependent alterations...
of neurocytes histopathology, which may be the cause of brain dysfunction after its prolonged use. The present study illustrated that TH could induce neurotoxic effects, in part, through alteration of p53, Bax, and Bcl-2 apoptotic pathway homeostasis. However, these findings were markedly reversible at the end of recovery period. Hence, these toxic possibilities should be taken into consideration when chronic use of TH, especially in large doses, is indicated. The delay in regression of neuropathology to normal after TH withdrawal necessitates more appropriate restrictions and scheduling reevaluations of this medication to avoid its potential abuse and dependence by medical and non-medical (street) addict individuals.

Acknowledgement:

The technical assistance of Prof. Dr. Taghreed A. Abd El Azez, Assistant Prof. of Pathology, Faculty of Medicine, Benha University, is deeply appreciated.
Table (1): The effects of TH on the apoptotic indices) A 1000 cells/slide was analyzed) of cerebral cortex and hippocampus neuronal cells in albino rats.

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<th>Cerebral Cortex</th>
<th>p53</th>
<th>Bax</th>
<th>Bel-2</th>
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<td></td>
<td>Mean±SD</td>
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<tr>
<td>Control</td>
<td>1.1±0.8</td>
<td>1.3±0.9</td>
<td>32.7±7.2</td>
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<td>Low TH</td>
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<td>16.2±6.8</td>
<td>17.4±3.3</td>
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<td>High TH</td>
<td>21.3±7.1</td>
<td>24.7±9.9</td>
<td>12.9±2.8</td>
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<td>Follow-up</td>
<td>9.8±4.4</td>
<td>8.7±4.5</td>
<td>24.5±5.1</td>
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<td>Follow-up vs Low</td>
<td>9.8±4 vs 15.2±5.6</td>
<td>8.7±4.5 vs 16.2±6.8</td>
<td>24.5±5.1 vs 17.4±3.3</td>
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<td>Follow-up vs High</td>
<td>9.8±4 vs 21.3±7.1</td>
<td>8.7±4.5 vs 24.7±9.9</td>
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<td>High TH</td>
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Significance: * = P<0.05, ** = p<0.001, *** = p<0.0001
Figs. (1-6): Histomorphological changes of CC. (1) Section of control rat brain showing the typical layered appearance of the CC labeled I-VI as follows: I-Molecular layer; II-External granular layer; III-Pyramidal cell layer; IV-Internal granular layer; V-Ganglionic layer; VI-Multiform layer. G = gray and W = white matters. (2-3) Degenerated neurons (→ and D) with dilated blood vessels (DV), apoptotic cells (➤), slight vacuolation of neuropil (*) and few red neurons (→) induced by low TH-dose. (4-5) Marked neuronal cell degeneration and disorganization as well as increased apoptotic cells (➤), extensive neuropil vacuolization (*) with marked, large numbers of red neurons (→), congested vascular channels (CV), hypercellularity, and inflammatory cell infiltrations (IC) produced by high TH-dose. (6) Less marked degenerative changes as shown in CC of follow-up group.
Figs. (7-10): HC histomorphological changes. (7) Normal HC neuronal cells (→ and *). (8) Some neurons showing degenerative changes (➤) with pyknotic nuclei and scanty eosinophilic cytoplasm (→) induced by low TH-dose. (9) Marked neuronal degeneration (→) is evident with prominent perineuronal retraction spaces and pronounced red apoptotic neurons produced by high TH-dose. (10) Less degenerative changes (→) in HC cells of follow-up group. All Fig. OM x 200.
Figs. (11-18): p53 IHC brown staining of CC and HC neurocytes nuclei. Marked expression of positive apoptotic cells in the both TH-treated groups compared to follow-up and control groups with CC being more affected than HC (OMx100). Small window showing +ve immunostained p53 neurocytes OMx400.
Figs. (19-26): Bax IHC brown staining of CC and HC neurocytes cytoplasm. Marked expression of positive apoptotic cells in the both TH-treated groups compared to follow-up and control groups with CC being more affected than HC (OMx100). Small window showing +ve immunostained Bax neurocytes OMx400.
Figs. (27-34): Bcl-2 IHC brown staining of CC and HC neurocytes perinuclear membranes. Less positive immunoreactivity of apoptotic cells in the both TH-treated groups compared to follow-up and control groups with CC being more affected than HC (OMx400).
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دراسة هستوباثولوجية وهستوكيميائية مناعية لنسبة مع الجرذان البيضاء 
بعد تعرضها للجويد الطويل لعقار الأمادول (ترامادول هيدروكلوريد)

المشتركون في البحث

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يرامادول هو دواء مخلل من أنبوبية الألبيونات ذو مفعول مسكن قوي عن طريق التأثير على الجهاز العصبي المركزي مباشرة وكذلك فإنه يستخدم في تسكين الألام المتوسطة والشديدة مع إحتفاظه بيئة كونه أقل في الأعراض الجانبية التي تسببها الألبيونات. يهدف هذا البحث إلى دراسة التأثيرات الفضائية للجرعات المختلفة لترامادول عند تناوله لمدة طويلة وأيضاً دراسة مدى تراجع هذا التأثير إذا ما توقف تعاطي الدواء، لذا

4 أسابيع، وقد أجريت هذه الدراسة على 20 جرذان بيئة كونه على جرعة الثانوية (مجموعة الجرعة الصغيرة) وتم معالجته بجرعة 50 مجم / كجم / يوماً عن طريق الفم، المجموعة الثالثة (مجموعة الجرعة الكبيرة) وتم معالجته بجرعة 100 مجم / كجم / يوماً عن طريق الفم. وذلك لمدة أربع أسابيع أما المجموعة الرابعة (مجموعة النائمة) فقد تم معالجتها مثل المجموعة الثالثة ثم توقف إعطاء الدواء لمدة أربع أسابيع قبل ذبحها. وفي نهاية مدة البحث تم دراسة نسبية المخلل في خلايا الدم والنواة، وانحلل الجرذان على مستوى القرشة المنقارية والهيموكروميس (قرن آمن) لاحظ أن أنغطا كانت أكثر شدة في الجرذان التي تم معالجته بالجرعة الكبيرة مقارنة بالجرذان التي عولجت بالجرعة الصغيرة وعند مقارنتهما بالمجموعة الضبائية التي لم تناو أي تغييرات هستوباثولوجية، أما المجموعة النائمة فقد أظهرت خلايا ملحوظاً في خلايا الدم والنواة تأثيرات أشد بعد تراجع معظم التغييرات البالغة التي حدثت نتيجة تعاطي الtramadol.

بإجراء الدراسة الهستوباثولوجية المناعية لنسبة مع الجرذان لكل من المجموعة الثانية والثالثة أظهرت تغييرات مناعية خلوية كيميائية على هيئة زيادة متردة ورود دالة إحصائية في كل من الف (Bcl-2) وال (Bax) ونقض ذو دالة إحصائية في كل من الف (p53) والأوزن (وار). وقد وجد أن هذه التغييرات تزيد كميات زائدة للجرعة، أما فيما يخص مجموعة المتابعة فقد حدث تراجع ملحوظا في صفات البروتينات عند مقارنتها بالمجموعة الثانية والثالثة بعد بعض الاختلافات عن المجموعة الضبائية. أما عن نسبة مع الجرذان تزيد زيادة الجرعة وذلك جزئياً عن طريق إحداث تنشيط لمرو الخلايا العصبية المبرمج كما أن هذه التغييرات قد أظهرت تحسناً عند توقف الدواء.