Histopathological Changes in Brain Tissues associated With Oral Administration of Tramadol in Male Rats

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Abstract

Background: Repeated and long treatment with tramadol might lead to accumulation of toxic metabolites in the body and increase the risk for pharmacokinetic interaction and decrease the clearance of tramadol, therefore this study was performed to investigate the toxic impact of the tramadol on the tissues of the brain in the male rats.

Method: The experiment was carried out at Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt. Thirty-two Albano Waster male rats weighing (200-250 g) were obtained from the animal house of the Faculty of Medicine, Alexandria University, and grouping into four groups (8 rats for each group in each cage). The Control group was fed a basal diet and given tap water daily for ten days. In group two the rats were fed basal diet and given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Ten days. In group three the rats were fed with basal diet and given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Twenty days. The group for the rats was fed with basal diet and given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Thirty days. At the end of the experimental period Kidney tissues of each rat were immediately removed and after weighted put into 10% neutral buffer formalin as a fixative solution and stained with Hematoxylin – Eosin stain.

The Results: The results observed a significant decrease in the weight of the brain in the groups of the rats that were given the Tramadol HCL in dose 45mg/kg B.W with increasing the time of administration as compared with the control group. Histopathological changes were observed in rats brain tissues section the rats that given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline for ten days revealed a mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels, and the tissue sections of group two after ten days revealed a mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels, while the three groups observed an increase in the vacuolar degeneration with neural atrophy and degeneration of neurons with reduction the neural process and pyknosis of the nuclei dilatation of blood vessels after twenty days of tramadol administration. The tissue sections obtained from group four after thirty days revealed an increase in the vacuolar degeneration, with more atrophy of the neural cells and complete reduction of the neural process and pyknosis of the nucleus in the injured neural cells and gliosis.

The conclusion of this study there are harmful toxic effects when administrated tramadol for long period on the brain tissues, therefore an abuse of tramadol should be avoided except with medical prescription owing to its toxic effects.

Key words: Tramadol HCL, Histopathological changes, Brain tissues
Introduction

Clinically the tramadol has been used for relieving mild and moderate pain in human and veterinary medicine and used as anesthesia especially in veterinary medicine. Tramadol is marketed as the hydrochloride salt and is available in a variety of pharmaceutical formulations for oral (tablets, capsules), sublingual (drops), intranasal, rectal (suppositories), intravenous, subcutaneous, and intramuscular administration. It is also available in combination with acetaminophen (paracetamol), as immediate- and extended-release formulations, and for once-a-day dosing described variously as ‘controlled’, ‘sustained’, or ‘delayed’ release.

The tramadol is rapidly absorbed orally and 30% of tramadol excreted through the kidney with half-life elimination (5-6) hours, while the remaining dose is metabolized in the liver. Tramadol in the liver is converted to O-desmethyl-tramadol by cytochrome P 450 which is an active substance and is two to four times more potent than tramadol. Persistent tramadol administration might lead to the accumulation of toxic metabolites in the body, increase the risk for its toxic kinetics effects, and/or lower the clearance of tramadol, thus increasing its potential for toxicity. The most common mechanisms of death after tramadol overdose are cardiorespiratory depression, resistant shock, asystole, and liver failure. Fatal toxicity of tramadol has been reported after coadministration of other medications including propranolol, ethanol, barbiturates, and benzodiazepines.

Repeated administration of tramadol might lead to toxic metabolites in the body and cause many adverse effects such as headache, constipation, nausea, dizziness, and central nerve disturbances. Neurotoxicity of tramadol has been reported in patients administrated tramadol both at the recommended dosage and the high dosage ranges in animal and human studies. The neurotoxicity of tramadol commonly manifests as generalized tonic-clonic seizures. Chronic use of tramadol in increasing doses causes neuronal degeneration in the rat brain, which probably contributes to cerebral dysfunction.

Many types of research were performed to detect the biochemical and histopathological changes due to long-term abuse the tramadol on the liver, kidney, and brain, also some studies dealt with abnormal histological changes in the testis.

The study was performed by Atici et al. (2005) founds biochemical and histological changes in the rat’s liver with significantly higher serum Alanine aminotransferase (ALT), Aspartateamino transferase (AST), Lactate dehydrogenase (LDH), and creatinine, also severe congestion and focal necrosis in the hepatocytes.

Youssif et al. (2016) found hemorrhage and cytolysis in the hepatocytes of the liver with complete cell membrane degeneration with changes in testicular tissues and atrophy in the somniferous tubules accompanied with interstitial calcification after administration of different doses of tramadol in experimental rats for 60 days. Hafez et al. (2015) observed that the toxic effect of tramadol on the parenchymatous organs such as liver, kidney, and thyroid glands in rats after intramuscular injection in different doses (12.5mg, 25mg, 50mg, and 300mg /Kg B.W) respectively for two weeks.

Many researchers have evaluated the effects of chronic use of tramadol on many organs, for example, liver, kidney, testes, heart, and thyroid gland, and scanty data dealt with the effects use of tramadol on the brain, therefore the objective of this study was designed to evaluate the toxic impact of the tramadol on the tissues of the brain in the male rats.

Material and Methods

Tramadol (tramadol HCL) 200mg/Kg B.W Tablet (Indian origin), were purchased from the outer pharmaceutical, Missan, Iraq. Experimental animals: Thirty-two Albano Waster male rats weighing (200-250 g) were obtained from the animal house of the Faculty of Medicine, Alexandria University. Animals were handled following the principles of laboratory animal care as contained in the NIH Guide for laboratory animal welfare and the experimental protocol was approved by the Local Ethics Committee and Animals Research.
rats were housed in stainless steel bottomed wire cages after grouping into four groups (8 rats for each group) and maintained at a temperature of 22 ± 2°C, relative humidity of 40-60%, with a 12 h/12 h light/dark cycle and allowed free access to food and water, the test substances were administrated to the animals according to the following experimental protocol:

· Group I: Control rats were fed basal diet and given tap water as drinking water daily for Ten days.

· Group II: Rats were fed basal diet and given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Ten days.

· Group III: Rats were fed with basal diet and given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Twenty days.

· Group IV: Rats were fed with basal diet and given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Thirty days.

At the end of the experimental period, the rats were overnight fasted (control and experimental animals) and sacrificed after 24 hours of the last dose of different administration under light ether anesthesia. Brains tissues of each rat were immediately removed taking care to handle specimens gently to minimize trauma, weighted, and put into 10% neutral buffer formalin as a fixative solution. Fixation time was limited to 24 hours and the fixed tissues were stored in 70% ethyl alcohol until they were processed. The fixed tissues were dehydrated through a graded series of ethanol and embedded in paraffin, sectioned according to the Luna (1968) method for histopathological examination, and stained with Hematoxylin –Eosin stain.16

**Statistical Analysis**

Statistical analyses were made with one-way analysis of variance (ANOVA) to compared the experimental groups(SPSS for windows version 17). P < 0.05 was considered statistical significance.

**The Results**

Table (1) Relative Brain weights(G) of the control group and Tramadol groups in different periods of the experimental protocol

<table>
<thead>
<tr>
<th>Exp rats</th>
<th>Control group</th>
<th>Group II(Tramadol within 10 days)</th>
<th>Group III(Tramadol within 20 days)</th>
<th>Group IV(Tramadol within 30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.53±0.63</td>
<td>1.44±0.07</td>
<td>1.33±0.47</td>
<td>1.23±0.55</td>
</tr>
<tr>
<td>2</td>
<td>1.55±0.62</td>
<td>1.43±0.72</td>
<td>1.38±0.55</td>
<td>1.35±0.64</td>
</tr>
<tr>
<td>3</td>
<td>1.65±0.53</td>
<td>1.41±0.77</td>
<td>1.32±0.52</td>
<td>1.26±0.66</td>
</tr>
<tr>
<td>4</td>
<td>1.56±0.33</td>
<td>1.51±0.61</td>
<td>1.35±0.57</td>
<td>1.30±0.58</td>
</tr>
<tr>
<td>5</td>
<td>1.72±0.48</td>
<td>1.53±0.80</td>
<td>1.41±0.49</td>
<td>1.27±0.56</td>
</tr>
<tr>
<td>6</td>
<td>1.68±0.56</td>
<td>1.63±0.73</td>
<td>1.32±0.51</td>
<td>1.37±0.63</td>
</tr>
<tr>
<td>7</td>
<td>1.61±0.51</td>
<td>1.47±0.13</td>
<td>1.41±0.11</td>
<td>1.25±0.33</td>
</tr>
<tr>
<td>8</td>
<td>1.71±0.21</td>
<td>1.45±0.23</td>
<td>1.39±0.08</td>
<td>-*</td>
</tr>
<tr>
<td>Total</td>
<td>13.01</td>
<td>10.36</td>
<td>10.91</td>
<td>9.03</td>
</tr>
<tr>
<td>Mean +SD</td>
<td>1.62± 0.48</td>
<td>1.29± 0.50</td>
<td>1.36± 0.04</td>
<td>1.12± 0.49</td>
</tr>
</tbody>
</table>
Table (1) showed the weight of the Brain obtained from the rats in the experimental groups, the results observed a significant decrease in the weight of the Brain in the groups of the rats that given the TramadolHCL in dose 45mg/kg .B.W with increasing the time of administration compared with the control group.

Histopathological changes in the brain:

Group I (Control group): Microscopic examination of brain tissue sections observed normal cerebral cortex with a normal distribution of neurons and fibers (N) in the neuropit, also the glial cells (GC) and oligodendrial cells (ODC) were found in normal structure (Fig 1).

Section of group (II) which represent the rats that given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline for ten days revealed a mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels (Fig 2).
Section of group (III): Observed the rats that given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline for Twenty days characterized by an increase in the vacuolar degeneration, with neural atrophy and degeneration of neurons with reduction the neural process and pyknosis of the nucleidilatation of blood vessels (Fig 3).

Figure (2): High power micrograph of rat brain section of group ( II ) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels.

Figure (3): High power micrograph of rat brain section of group ( III ) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels, NCA: Neural Cell Atrophy, NP: Neural Process.
Section of group (IV): Which represent the rats that given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline for Thirty days revealed an increase in the vacuolar degeneration, with more atrophy of the neural cells and complete reduction the neural process and pyknosis of the nucleus in the injured neural cells and gliosis (Fig 4).

Figure (4): High power micrograph of rat brain section of group ( IV ) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels, NA: Neural Atrophy, NP: Neural Process.

Discussion

Oxidative stress, an imbalance between oxidant and antioxidant mechanisms in animal bodies, this imbalance may result in either form excessive exposure to pro-oxidants or from compromised anti-oxidant mechanisms. Which may result from deficiency of essential elements or incapacitation of disease, while the former might emanate from exposure to exogenous toxins or the pathologic stress of disease17,18.

Tramadol hydrochloride used as analgesic drugs, therefore in the 70s used for treating moderate and severe pain but in recent years the tramadol abuse among youngers and teens in different countries especially between males, therefore the present study performed to investigate histopathological changes in the brain tissues accompanied with the toxic effect of tramadol in male rats.

The result revealed a significant decrease in the weight of the brain in a group of rats that administrated tramadol at different times as compared with the control group, this result agrees with Balhara et al (2018) that founds the administration of tramadol caused a reduction in the cells volume and nuclear condensation in the brain of rats which probably contributes to cerebral dysfunction19.
In present study observed varied adverse effects in morphological and histological structures of the brain tissues with increasing the time of given dose of tramadol. Essam et al (2015) found histological changes in the brain tissues of rats after continuous administration of tramadol for a long period20.

Mohamed et al (2013) found changes in the pyramidal cells which lost the shape and increase the hemorrhage in the brain and disrupted ependyma and the choroid plexus become hypertrophoid21.


Chronic administration of tramadol with increasing the doses of the drug may cause degeneration in the red neurons and apoptosis in the brain which contributes to cerebral dysfunction (Atici et al, 2005)23.

Some researchers have reported the adverse effect of tramadol on other organs in the body, where Azari et al (2014) referred the long term administration of tramadol can cause to the testicular tissues and deposition of acidophilic PSA-positive materials in male rats24. Youssef and Sheweita et al (2018) reported that administration of morphine and tramadol can cause degeneration in the hepatocytes and dilatation in the central vein with dilatation in the sinusoid25.

The study was performed by Abou Eluaga et al (2020) to investigate the effect of tramadol on the histological structures of the testes in Albano rats which observed abnormal changes in the seminiferous tubules with long-term administration of tramadol11.

Salma et al (2003) referred that tramadol may increase the accumulation of free radicals and ROS which can cause an increase in nitric oxide level in the brain and lead to hypofunction of Leydig cells with consequent reduction of the testosterone secretion26.

Caju et al (2012) reported that exposed the mature rats to high doses of tramadol and morphine for a long time can cause testicular changes due to disorders in the endocrine and paracrine functions with reduction of Sertoli and Leydig cells leading to disorders in LH, estradiol, somatotropin, somatostatin, and gonadotrophin-release hormone27.

Hussein et al (2017) recorded an increase in the area and creatinin levels in rats after received tramadol (22.5 mg/Kg B.W/day for nine weeks) due to evidence of renal damage and impaired renal function28.

In conclusion, the results of this study observed a harmful toxic effect on the histological structures and function of the brain in male rats when administrated the tramadol for long period, therefore abuse of tramadol should be avoided except with medical prescription owing to its toxic effects.

**Ethical Clearance**- Taken from Farmacy college/ Misan University/Ethical committee

**Source of Funding**- AUTHORS OWN MONEY ONLY

**Conflict of Interest** – NIL

**References**


