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 that could cause toxic metabolites by accumulation in the body and with high level of risk of pharmokinetic interaction alongside the decreasing of tramadol, therefore this study was carried out to investigate the toxic impact of the tramadol on the tissues of the brain in the male rats.

Method: This experiment was accomplished and processed at environmental toxicology laboratory, environmental studies department, institute of graduate studies and research, university of Alexandria, Alexandria p) ovince, Egypt. *a group of rats (32, male) from Albano waster subspecies and they weigh from 200-250g, all belonging to the animal house of the faculty of medicine, university of Alexandria* and grouping in to four groups (*8 rats for each group* in each cage). In regards to the control group, they were given basal food and tap water through-out 10 days by gastric tube, day by day. 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. Group for 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 Thirty days. When the experimental period reached its end, kidney tissues of each rat were instantly removed and after that weighed then put into 10 percent of neutral buffer formalin to be considered as a fixative solution.

The Results: The results showed significantly decrease in the heaviness of the brain in the groups of the rats that given the Tramadol HCL in dose 45mg/ kg .B.W with increasing the time of administration as compared to 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 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 mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels, while the three group Observed 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 the obtained from the group four after thirty days revealed 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 glyosis.

The Conclusion of this study there are harmful toxic effects when administrated the tramadol for long period on the brain tissues, therefore 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 widely used in hum and veterinary medicine for relieving mild and moderate pain[1]. Tramadol is a known hydrochloride salt and can be found in different pharmaceutical formulations and many applications for example; oral consumption like

capsules or tablets, or in form of drops for intranasal, subcutaneous, rectal, intravenous, and intramuscular administration, on the other hand it also exists in combination with acetaminophen (paracetamol) to be used as immediate or extended formulations [2].

The tramadol rapidly absorbed orally and 30% of tramadol excreted through the kidney with half – life elimination (5-6) hours, while the remaining dose metabolized in liver. Tramadol in the liver is converted to O-desmethyl-tramadol by cytochrome P 450 (an active substance) and is 2-4 times more powerful than tramadol[3,4] (Dickman, 2007; Khandaved *et al* ,2010). Accumulation of toxic metabolites in the body might be caused by tramadol administration leading to increasing the risk of its toxic kinetic effects and the clearance of tramadol becomes low, making its potential toxicity higher.[5] The most common forms of death that can combine with tramadol overdose like asytole, liver failure, resistant shock and cardiorespirating depression, furthermore the tramadol and its fatal toxicity was also reported after coadministration of other medications such as ethanol, barbiturates, benzodiazepines and proprano LOL[6]. Repeated administration of tramadol may cause toxic metabolites in the body and cause many adverse effects such as headache, constipation, nausea, dizziness, and central nerve disturbances[7]. 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[8]. The tramadol neurotoxicity is commonly manifested to being general tonic-clinic seizures. Severe tramadol use in increased doses which results in neuronal deterioration in the brain of the rat, which could contribute to cerebral dysfunction[9].

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[10,11].

The study was performed by Atici *et al* (2005) founds biochemical and histological modifications in the liver of the rats with noticeably increased serum Alanine aminotransferase(ALT), Aspartate aminotransferase(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 administrating various doses of tramadol in experimental rats for 60 days[12]. 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.[13]

The large effect of chronic use of tramadol in many body organs like thyroid, liver and kidney have been evaluated and reported by many researchers 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.[14,15]

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) in regards to the experimental animals, they were acquired from Alexandria University / Medicine College. After their arrival they were taken care of according to animal care principles from NIH guide to animal laboratories. The experimental procedure that was used had been approved by local ethics committee and animal research. A cage was made from stainless steel bottomed wire was used for housing the rats.

- Group I: for ten days rats were fed with a basal diet and tap water respectively

- 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.

Finally, the rats were fasted overnight (control and experimental animals) and sacrificed after 24 hours of the last dose of different administration under light ether anesthesia. Brain tissues of each rat directly removed gently and with good attention specimens handling to minimize trauma and then weighted to be ready for mixing with formalin (neutral buffer) as a fixative solution. 24 hours was the time used as fixation time the to be stored in 70% ethyl alcohol for the fixed tissues until they were processed. The next step was the dehydration of fixed tissues by using 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

Exp rats	Control group	Group II(Tramadol within 10 days)	Group III(Tramadol within 20 days)	Group IV(Tramadol within 30 days)
1	1.53+ 0.63	1.44+ 0.07	1.33+ 0.47	1.23+ 0.55
2	1.55+ 0.62	1.43+ 0.72	1.38+ 0.55	1.35+ 0.64
3	1.65+ 0.53	1.41+ 0.77	1.32+ 0.52	1.26+ 0.66
4	1.56+ 0.33	1.51+ 0.61	1.35+0.57	1.30+0.58
5	1.72+ 0.48	1.53+0.80	1.41+0.49	1.27+0.56
6	1.68+ 0.56	1.63+0.73	1.32+0.51	1.37+0.63
7	1.61+ 0.51	1.47+0.13	1.41+0.11	1.25+0.33
8	1.71+ 0.21	1.45+0.23	1.39+0.08	-*
Total	13.01	10.36	10.91	9.03
Mean +SD	1.62+ 0.48	1.29+ 0.50	1.36+ 0.04	1.12+ 0.49

*Dead rat.

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 Tramadol HCL in dose 45mg/ kg .B.W with increasing the time of administration in compared to the control group.

Histopathological changes in the brain:

Group I which is the control group, microscopic examination of brain tissue sections observed normal cerebral cortex with a regular distribution of neurons and fibers (N) in the neuropil, also the glial cells (GC) and oligodenderial cells (ODC) were found in normal structure (Fig 1).

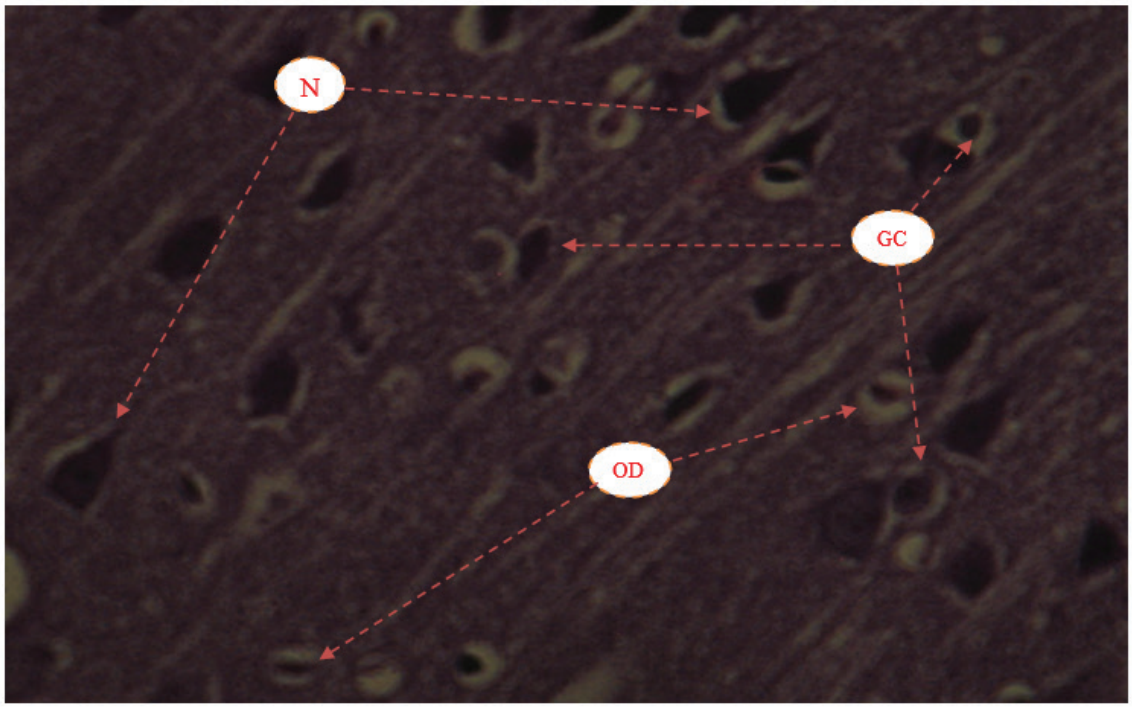

Figure (1): High power micrograph of rat brain section in control group stained with Haematoxylin & Eosin (H&E, X400), N: neural cells, GC: glial cells, OD: oligodendroglial cells.

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).

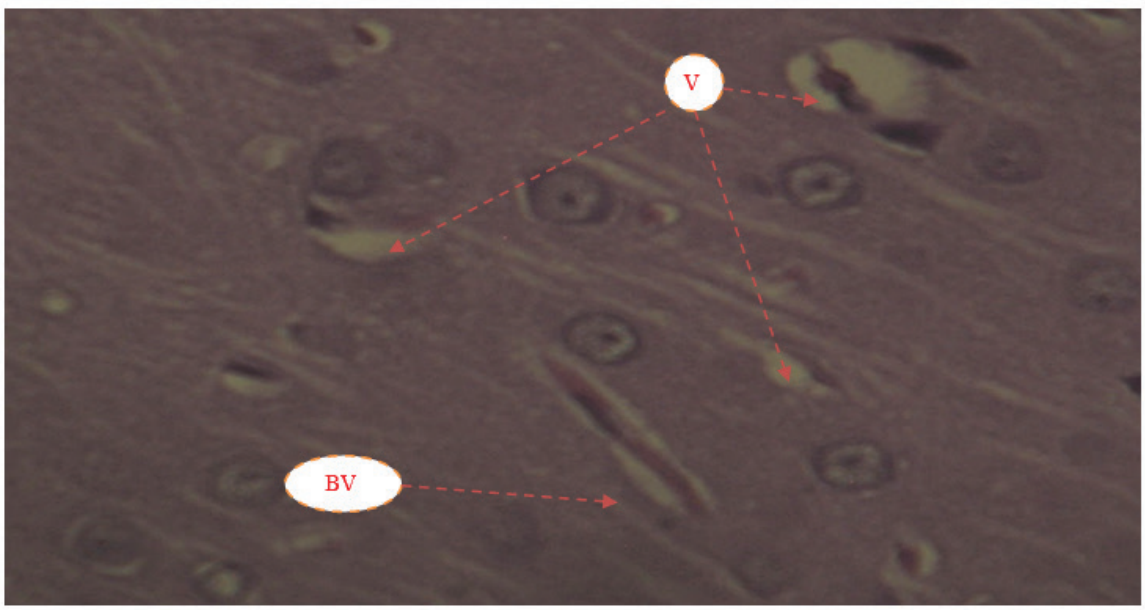

Figure (2): High power micrograph of rat brain section of group (II) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels.

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 nuclei dilatation of blood vessels (Fig 3).

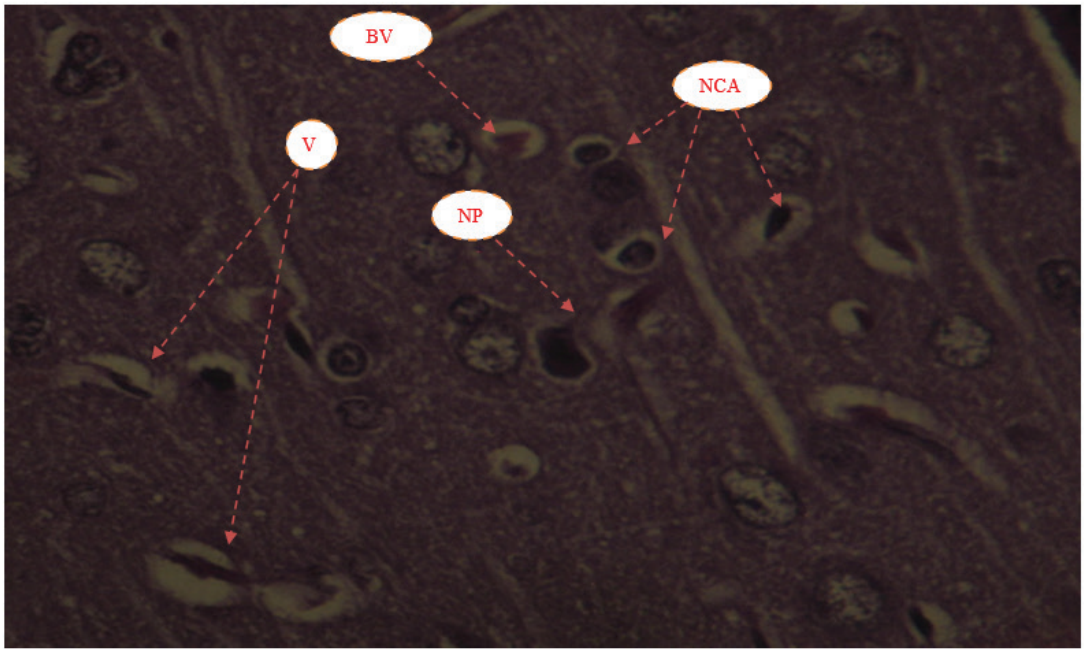

Figure (3): High power micrograph of rat brain section of group (III) stained with Hematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels, NCA: Neural Cell Atrophy, NP: Neural Process.

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).

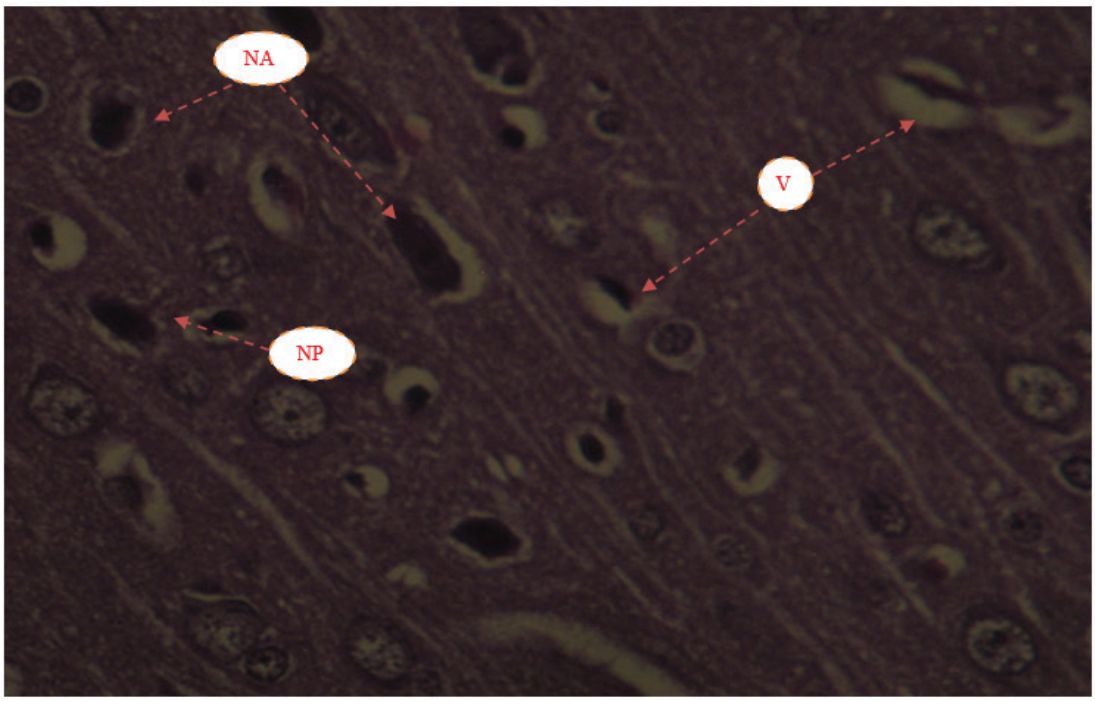

Figure (4): High power micrograph of rat brain section of group (IV) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels, NA: Neural Atrophy, NP: Neural Process.

Discussion

Oxidative stress, which is an inequitable relationship between both oxidant and antioxidant mechanisms throughout animal bodies, the result of this inequity may be in either form too much interaction with pro-oxidants or from compromised anti-oxidant mechanisms. Possibly resulting in a incapacitation of disease or shortage of significant elements, whereas the incapacitation of disease may cause the former to emanate,[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 teenagers in different countries mostly between males, therefore the following study performed to investigate histopathological changes in the brain tissues accompanied with the tramadol toxicity in male rats

The result revealed a that a group of rats had a significantly lighter brain than before that administrated tramadol at different times when in comparison with the control group, the result is similar to that of 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 dysfunction[19].

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 period[20].

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 hypertrophoid[21].

Abou Elfatoh *et al* (2014) founds congestion of blood vessels and degeneration in the neural cells after chronic using of tramadol[22].

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

Some researchers have shown the large effect on other body organs, 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 rats[24]. 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 dilation in the sinusoid[25].

The study was performed by AbouEluaga *et al* (2020) to look into the consequences 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 tramadol[11].

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 secretion[26].

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 endocrine and paracrine function disorders. While reducing of both Sertoli and Leydig cells leading to disorders in LH, estradiol, somatotropin, somatostatin, and gonadotrophin- release hormone[27].

Hussein *et al* (2017) recorded an increase in the area and creatinine 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 function[28].

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 an 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

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Conflict of Interest – NIL

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