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Does Tramadol Exposure Have Unfavorable Effects on Hippocampus?: A Review Study

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Abstract

Introduction: Tramadol, one of the most common opioid pain relievers, acts upon the μ-receptor in the central nervous system (CNS) to alleviate pain associated with various situations like postoperative pain, arthritis, and muscular pain. Additionally, it has been utilized to address depression and anxiety disorders. Extensive research has shown that tramadol can potentially inflict irreversible harm on different regions of the CNS, including the cerebrum, cerebellum, amygdala, and, notably, the hippocampal formation. However, the precise mechanism behind these effects remains unclear. Within this study, we conducted a comprehensive examination of the impacts of tramadol on the CNS, specifically focusing on hippocampal formation.

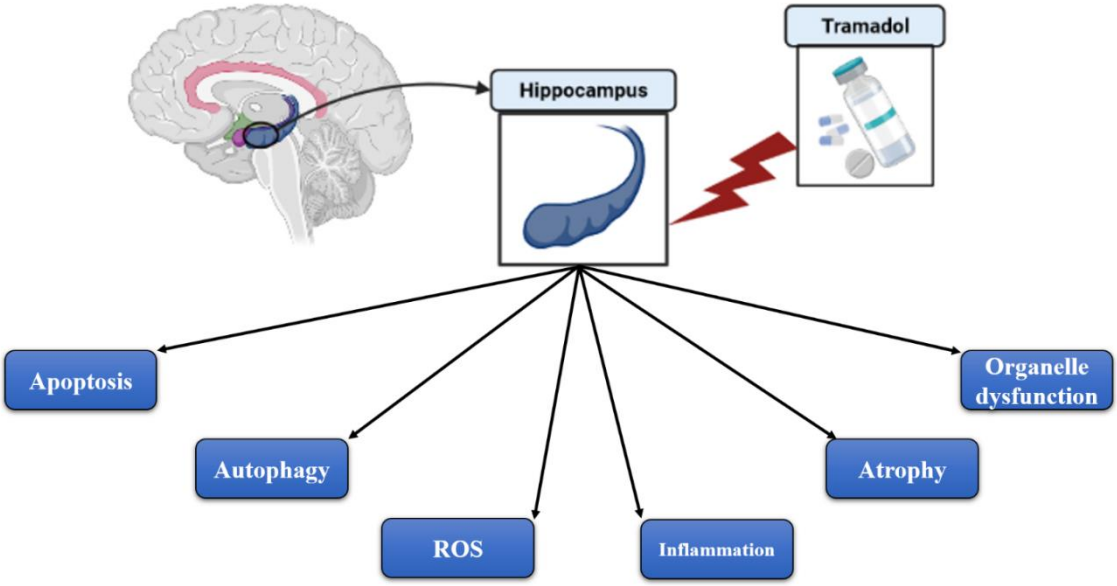
Method: In this study, we collected relevant articles published between 2000 and 2022 by conducting searches using specific keywords, including Tramadol, Tramadol Hydrochloride, Central Nervous System, Hippocampus, and Hippocampal Formation, in various databases.

Results: The results of this study proposed several processes by which tramadol may impact the CNS, including the induction of apoptosis, autophagy, excessive production of free radicals, and dysfunction of cellular organelles. These processes ultimately lead to disturbances in neural cell function, particularly within the hippocampus. Furthermore, it is revealed that tramadol administration led to a significant decrease in the neural cell count and the volume of various regions within the brain and spinal cord.

Conclusion: Consequently, neuropsychological impairments, such as memory formation, attention deficits, and cognitive impairment, may happen. This finding highlights the potential impacts of tramadol on neural structures and warrants further investigation.

Keywords: Tramadol, Central nervous system, Hippocampus

Graphical Abstract



1. Introduction

Today, numerous studies have reported that many drugs used in the treatment of various diseases, particularly analgesic drugs, in addition to their therapeutic properties, are unfortunately misused in an illegal way, which not only can cause various irreparable side effects on different body systems but also impose enormous costs on healthcare systems. Hence, recently, drug abuse has become an essential social matter worldwide.[1, 2]

Tramadol is known as 2-(dimethylamino)-methyl)-1-(3′-methoxyphenyl) cyclohexanol hydrochloride. The analgesic effects of tramadol hydrochloride (TH) result from a dual mechanism of action, which involves both opioid and non-opioid properties. TH acts on opioid receptors (μ,κ) with low affinity, exhibiting a frail agonist effect. Furthermore, it influences monoamine receptor systems by impeding serotonin (5-HT) and norepinephrine (NE) reuptake, suppressing pain transmission in the spinal cord.[3, 4] This drug was first discovered and synthesized in 1962 by a German company (Grünenthal GmbH) for pain treatment and brought to market in 1977 under the name "Tramadol."[4] Tramadol is a commonly prescribed opioid pain reliever with the molecular structure of C_{16}- H_{25}-NO_2.[5] Nowadays, TH is administered through various routes, including oral, intravenous, intramuscular, sublingual, and subcutaneous.[6] TH undergoes primary metabolites in the liver, mainly mediated by cytochrome P450 isoenzymes. The primary metabolites of TH are O-desmethyltramadol (M1) and N-desmethyltramadol (M2).[4] All metabolites undergo conjugation with glucuronic acid and sulfate, before being expelled in urine.[6, 7] Narcotic drugs are widely used for their powerful analgesic properties. These drugs, such as morphine, fentanyl, oxycodone, methadone, and

tramadol, usually exert their analgesic effects through reaction with μ receptors in the nervous system.[4, 8, 9] Clinical results have demonstrated tramadol's a good potential for pain relief; however, its analgesic properties are inferior to morphine and codeine.[6, 10, 11] Therefore, the use of TH could be a viable option to relieve pain in pathological situations such as post-surgery, fibromyalgia, arthritis, cancer, and muscle pain.[12, 13] Unfortunately, physical and psychological dependence on this drug is widely reported around the world today, and thus, it has a high abuse potential.[5, 14, 15] Nowadays, the illegal use of tramadol has become increasingly prevalent worldwide, which could result in significant healthcare costs for governments. The incidence rate of tramadol abuse varies across countries and is estimated to be 69 per 1000 individuals annually, while the annual rate of dependence is approximately 6.9 per 1000 individuals.[16] Tramadol abuse can lead to poisoning and death, which can occur due to overdoses, long-term abuse, or interactions with other drugs.[2] Therefore, tramadol has been listed as a controlled substance around the world due to its potential for abuse and dependence.[16] Tramadol can impact various organs and tissues, giving rise to indications such as 5-HT syndrome, seizures, liver conditions like cirrhosis, impaired kidney function, increased intracranial pressure, non-mental nervous disorders, respiratory depression, cardiovascular disorders, and gastrointestinal issues[2, 17] A bulk of studies have shown tramadol's negative effects on the various parts of the brain, such as the prefrontal cortex, cerebellum, amygdala, and hippocampus.[3, 5, 18]

It is well-documented that tramadol could induce significant changes in the noradrenergic and serotonergic systems in the central nervous system (CNS).[19, 20] It also inhibits NE and 5-HT reuptake in the CNS; for this reason, some studies have suggested that this drug may have beneficial effects on reducing symptoms of anxiety, phobias, and depression disorders.[21, 22] Despite the curative effects of tramadol, various side effects, such as vomiting, itching, constipation, hyponatremia, apnea, tremors, and tachycardia, have also been reported [23-25].

According to the results of some recent investigations, it is evident that TH could obviously have several adverse impacts on the different human body systems, such as cardiovascular, gastrointestinal, respiratory, renal, and endocrine systems, as well as the CNS.[6, 9, 20] For instance, tramadol is broken down by hepatocytes and then is removed from the body through the urinary system. Therefore, uncontrolled use of tramadol can disrupt the normal functions of liver and kidney cells, ultimately impairing their normal physiological functions.[9] In an animal study, Elkhateeb et al. (2015)revealed a considerable elevation in aspartate transaminase (AST) and alanine transaminase (ALT) as hepatic enzymes, and creatinine in tramadol-treated rats.[26]

Due to the direct impact of tramadol on the nervous system by reacting with the μ opioid receptor (MOR) and affecting the serotonergic and noradrenergic systems, many studies have been conducted to discover the destructive effects of this drug on the CNS.[4, 21] Some previous studies have shown that using TH could significantly increase the risk of seizures.[27, 28] Researchers have proposed that tramadol may induce seizures by affecting gamma-aminobutyric acid (GABA)-ergic, dopaminergic, and histaminergic neurons in the CNS.[27]

Multiple ultrastructural changes in different parts of the CNS have also been reported in tramadol-treated animals.[29, 30] In this regard, Omar et al. (2016) explored structural changes in tramadol-treated animals in the motor cerebral cortex. Their results revealed apoptotic ultrastructural features, such as nuclear membrane indentation, DNA condensation, disruption in mitochondrial cristae, Golgi apparatus expansion, rough endoplasmic reticulum (rER) dilation, and myelin sheath integrity disruption.[21]

Furthermore, another study indicated that morphine, an opioid analgesic with a structure similar to tramadol, could elevate the count of apoptotic cells in the spinal cord and cerebral cortex of morphine-administered rats compared to controls. Thus, receptors and caspase-3 (apoptotic marker) up-regulate markedly in chronic treatment with morphine.[31]. Furthermore, thehistopathological findings of Fathy et a.l's (2013) study demonstrated that TH caused a remarked increase in the number of dark neurons in the brain and spinal cord. It has also been reported that tramadol may impact endothelial cellular integrity, potentially leading to dysfunction of the blood-brain barrier (BBB).[32]

Besides, several studies have illustrated that analgesic drugs that act through opioid receptors, such as buprenorphine and tramadol, could have adverse effects on neuropsychological performance, such as executive functions, attention, and cognitive flexibility.[33, 34] Recent studies have also shown that tramadol has negative impacts on the hippocampus, leading to impairments in learning, semantic memory, visual memory, and working memory.[25, 35-37]

While some studies suggest a potential impact on memory, there may be variations in study designs and outcomes. Further research is needed to fully understand the specific mechanisms through which tramadol may influence memory and cognitive function. Hence, in this review, we focused on various aspects of the effects of tramadol on the hippocampus.

2. Method

In this review study, we explored the articles that examined the effects of tramadol on the hippocampus. For this purpose, we used keywords such as Tramadol, Tramadol Hydrochloride, Central Nervous System, Hippocampus, and Hippocampal Formation to find related studies in this regard by searching PubMed, Google Scholar, Elsevier, Medline, and Scopus databases

between 2000 and 2022. Following the initial search, we identified relevant articles. All these articles underwent a thorough review, assessing their titles and abstracts. During this process, we eliminated duplicates and articles that did not align with our research objectives. Ultimately, we exclusively chose articles that specifically assessed the impacts of tramadol on the hippocampus.

3. Results

3.1. Effects of Tramadol on Memory

The CNS, particularly the brain, is a highly metabolically active part and very sensitive to various drugs and substances with neurotoxic potential.[13] It is well-documented in recent studies that these neurotoxic factors could exert their destructive effects on the CNS, particularly in the hippocampus, by producing high levels of reactive oxygen species (ROS), promoting apoptosis and autophagy, altering normal mitochondrial function, and inducing inflammation. Exposure to such medications during life induces alterations to brain functioning that may conduce to adverse and even lethal health consequences.[5]

The hippocampus has a C-shaped structure and is one of the key parts of the human brain located in the temporal lobe.[38] This structure is divided into four sub-regions: CA1, CA2, CA3, and dentate gyrus. All these regions receive information from specific parts of the brain.[39, 40] It is well known that the hippocampus plays a principal function in the formation of different types of memories (working, motor, semantic, episodic, or autobiographical memory), as well as spatial navigation and cognition.[36, 37, 41] As mentioned, tramadol induces its analgesic effect through direct interaction with various parts of the CNS.[4, 6] In addition, previous studies reported neuropsychological performance impairments after tramadol administration.[33, 34] However, the exact mechanism by which tramadol induces structural alteration in the CNS (particularly the hippocampus) is not entirely understood. Therefore, due to the use of this drug for its therapeutic properties and the increase in illegal uses, many studies have been carried out to understand the exact mechanism of its action on the nervous system.

3.2. Effects of Tramadol on Memory in Human Studies

Based on the results of these studies, researchers have proposed that tramadol could negatively impact attention, cognitive functions, executive functions, learning, and memory.[17, 42-46]

Research studies evaluating the tramadol side effects in humans have consistently shown that the abuse of this drug has destructive effects on various cognitive functions, such as the formation of different types of memory (work, visual, verbal, spatial), visual attention, learning, verbal knowledge, full-scale intelligence quotient (IQ), verbal IQ, performance IQ, and task switching. However, researchers have found that long-term abstinence from tramadol

use significantly improves cognitive test scores in tramadol abusers.[42, 45-47] Additionally ,a rare clinical report documented significant left hippocampal atrophy with abnormal morphology on magnetic resonance imaging (MRI) of the brain in a 20-year-old male who had utilized TH for three years.[48] Due to the importance of the topic, many studies have investigated the effects of tramadol on the nervous system in animal models.

3.3. Effects of Tramadol on Memory in Animal Studies

According to prior studies, tramadol administration could effectively disrupt normal neurotransmitter function in the CNS.[25, 49] As memory formation is a highly sophisticated process, and it is well-known that a wide range of neurotransmitters is involved in it, including GABA, 5-HT, and acetylcholine, it is believed that tramadol may affect memory formation.[15, 22, 50] Therefore, numerous studies have reccently been conducted in this context. In this case, Hosseini-Sharifabad et al. (2016) explored acute and chronic tramadol administration on spatial memory in rats. Their data showed significant impairments in spatial memory by utilizing the object recognition task (ORT) test. Interestingly, they reported that acute tramadol administration has more destructive effects on spatial memory than chronic administration. They hypothesized that tramadol might negatively impact memory by affecting various neurotransmitter systems and some secondary messengers, such as adenosine 3':5'-cyclic monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP).[51]

Multiple lines of evidence showed that the hippocampus, particularly the CA1 subfield, plays a vital role in memory formation and retrieval, and any damage to this area could lead to memory impairment.[39, 52, 53] Interestingly, a high level of opioid receptors has been detected in this part.[12, 22] In this regard, the results of Niknamfar et al.'s (2019) study showed that rats that received tramadol in an intra-CA1 injection manner revealed considerable impairment in memory formation and retrieval. Also, they reported that TH could induce state-dependent memory in rats. A previous study suggested that the acetylcholine receptor (muscarinic), expressed in hippocampal formations, might be involved in tramadol-induced state-dependent memory.[35] Moreover, the results of a prior study proposed that tramadol could increase acetylcholinesterase enzyme activity and drop Na^+/K^+/ ATPase function, leading to cognitive impairment.[54] As there are high levels of opioid receptors in the hippocampus, it is reported that TH may decrease second messengers (cAMP and cGMP) involved in memory processes, which could ultimately lead to memory impairment.[55, 56]

Moreover, according to Jafari-Sabet et al.'s (2016) study, intra-CA1 tramadol administration could considerably disrupt the retrieval of inhibitory avoidance memory. They used physostigmine (acetylcholinesterase inhibitor) and atropine (muscarinic acetylcholine receptor

antagonist) to investigate whether muscarinic acetylcholine receptors might affect memory formation. Their results illustrated that tramadol-treated animals that received physostigmine showed a marked improvement in memory retrieval. Moreover, they reported that atropine could inhibit tramadol state-dependent memory.[49]

Based on previous studies, it is accepted that the hippocampus (CA1 subfield) plays a fundamental function in memory formation.[57] Inotropic glutamate receptors, N-methyl-D-aspartate receptors (NMDARs), are broadly expressed in various parts of the CNS, particularly in the hippocampus.[58, 59] Some experimental studies showed that NMDAR antagonist-treated animals revealed significant impairment in memory formation. Hence, researchers have proposed that NMDAR is essential for synaptic plasticity and memorial functions.[60, 61] In addition, it is well-documented that this receptor and neural nitric oxide (nNO), which is highly expressed in the hippocampus, are involved in the learning and memory process.[62, 63] The results of an experiment conducted by Jafari-Sabet et al. (2018) showed that tramadol could induce amnesia, reversing this impairment after using L-arginine (an NO precursor) through CA1 NMDAR/NO signaling pathway.[64] Another investigation reported that Ca2+/calmodulin-dependent protein kinase II-cAMP responsive element binding protein (CAMKII–CREB) signaling pathways may be impacted by tramadol, ultimately leading to memory impairment.[60] Altogether and consistent with these results, researchers suggested that NMADRs in the hippocampus have pivotal roles in tramadol state-dependent memory regulation.[60, 64, 65]

3.4. Antidepressant Effects of Tramadol by Affecting the Hippocampus

Several prior studies have demonstrated that tramadol also has antidepressant effects. Researchers have attributed this property of tramadol to inhibiting the reuptake of the neurotransmitter 5-HT and NE.[21, 66, 67] In addition, it is indicated that the mammalian target of rapamycin (mTOR) has an essential function in the antidepressant effects of ketamine through the up-regulated mTOR in the prefrontal cortex.[68-70] Consistent with these results, it is well-documented that the mTOR expression level also decreases in depressed patients' brains.[71] A study by Yang et al. in 2012 indicated that pretreatment with tramadol could enhance the antidepressant potential of ketamine by increasing mTOR expression within the hippocampus and prefrontal cortex.[72]

In addition, another study conducted by Yalcin et al. (2007) examined the antidepressant-like effect of tramadol in male inbred BALB/c ByJ mice by using the unpredictable chronic mild stress model by applying the unpredictable chronic mild stress model (UCMS). The results of their study showed a significant elevation in the levels of noradrenaline (NA) and 3-methoxy-4-hydroxy-phenyl glycol (MHPG), the primary NA metabolite, in the hippocampus. Based on

their findings, researchers proposed that tramadol could induce its antidepressant-like effect by affecting the β-adrenoceptors (β2-subtype).[73]

3.5. Tramadol Effects on Hippocampus at Cellular Level and its Probable Mechanisms

An experimental study by Bloms-Funke et al. (2011) demonstrated a marked rise in the extracellular level of 5-HT and NA in the rat ventral hippocampus after tramadol treatment in a dose-dependent manner.[74]

Khatami et al. (2022) studied the effect of tramadol on the PC-12 cell line (as part of the in vitro study) and the hippocampus (as part of the in vivo study). The in vitro results of this study revealed that tramadol significantly increased the generation of ROS, apoptotic (TP53, P21), and autophagic (MAP1LC3B, BECN1, ATG12, LAMP2) and mTOR genes in PC-12 cells. For the in vivo part, the researchers treated animals with TH for three weeks (50 mg/kg). Their results demonstrated that tramadol could cause a dramatic elevation in glial activation and up-regulation of inflammatory and apoptotic markers. In addition, their data revealed that tramadol also had adverse effects on memory function, spatial learning, and long-term potentiation (LTP). Also, according to stereological assessments, they reported a notable decrease in the count of neural cells and volume of tramadol-treated rat hippocampus. They also evaluated the effects of tramadol on microglial function and activity. The immunohistological staining against Iba-1 (microglia proliferation marker) revealed that the number of Iba-1 positive cells increased. In contrast, the complexity of microglia cells decreased in the hippocampus of tramadol-treated animals.[5] Similar to the above study, Nafea et al. (2016) clearly established that tramadol had various adverse effects on the brain and hippocampus, such as causing spatial memory impairment, 5-HT levels elevation, increased stress oxidative production, and up-regulated caspase-3 gene expression.[15]

Another study also investigated the adverse effects of tramadol on the hippocampus of male albino mice. The author found histological and immunohistological changes in the hippocampus of mice receiving tramadol for 30 days through the gastric tube. They observed that these animals had neural degeneration in CA1, CA3, and DG sub-regions, a dramatic alteration in mitochondria and rER. Besides, their immunohistological results revealed that caspase-3, CD-68 (microglial marker), and glial fibrillary acidic protein (GFAP) (astrocyte marker) positive cells elevated significantly in all hippocampal sub-regions. Also, they reported a significant reduction in DG, CA1, and CA3 region thickness.[13] Other investigations indicated that TH could drop CA1 neuronal activity. Based on their finding, the authors proposed that this effect of tramadol might be mediated by endocannabinoid and orexin systems.[22]

It is documented that the count of dark neurons in the hippocampus increased in tramadol-treated animals, similar to the occipital and frontal cortex, proving the destructive effects of tramadol on different parts of the CNS.[75, 76]

In a recent in vitro study, Hosseindoost et al. (2022) examined the impact of tramadol exposure on hippocampal neurons' programmed cell death and synaptogenesis. They carried out a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to assess neural cell viability and a western blot technique to asses synaptogenesis (synaptophysin, stathmin) and apoptosis (caspase-3). Their results indicated that tramadol exposure reduced cell viability and synaptogenesis markers, whereas caspase-3 protein levels increased.[77]

El-Kerdasy et al. (2020) examined the effects of tramadol administration on the male albino rabbit hippocampus. They treated animals with an increased dose of tramadol (42, 84, and 168 mg/kg/day). The results of their study demonstrated a marked disarrangement of neural cells, intercellular space expansion, degenerative vacuolation, wide chromatolysis, and increased caspase-3-positive cells in the hippocampus.[76]

Another study also revealed that tramadol could disrupt spatial learning and memory, as well as mitochondrial function, as shown by elevated mitochondrial edema, matrix metalloproteinase (MMP) dysfunction, raised ROS levels, and cytochrome c release from mitochondria in the hippocampus.[78]

Another experimental study by Baghishani et al. (2018) also evaluated the impact of tramadol use on rats' hippocampus. The results of their investigation demonstrated that tramadol caused a raise in the apoptotic cell count, a significant impairment in memory, and a marked elevation in the number of dark neurons in the different sub-fields of the rats' hippocampus.[75]

In an experimental study, Ahmadian-Moghadam et al. (2021) found that the impact of tramadol exposure (acute and chronic) on opioid receptors (μ and δ) and p-CREB was evaluated in the hippocampus. Their data indicated that the levels of δ-receptor and p-CREB significantly increased in both acute and chronic tramadol exposure. Interestingly, the level of μ-receptor was reduced in acute exposure, whereas a marked elevation in chronic exposure was revealed.[79]

Additionally, the outcomes of a recent animal study have demonstrated that tramadol can induce neurotoxic impacts upon the CNS by disrupting equilibrium within the oxidant and anti-oxidant systems. In this context, authors treated albino rats with tramadol at diverse dosages (25, 50, and 100 mg/kg). Their obtained data revealed a significant elevation in malondialdehyde (MDA) and 8-hydroxy-2' -deoxyguanosine (8-OHdG) levels; also, a dramatic decrease in catalase (CAT), superoxide dismutase (SOD) activities, and glutathione

(GSH) content ultimately conduced to oxidative DNA damage within the CNS.[80] Some studies suggest that tramadol might influence glutamate signaling. Prolonged use or high doses could potentially lead to disruptions in glutamate balance, possibly contributing to excitotoxicity and neuronal damage.[81-84]

The results of a current study revealed that tramadol could lead to reduced levels of SOD and GSH in the cerebrospinal fluid (CSF). Additionally, stereological assessment demonstrated a notable modification in the volume of the choroid plexus (CP), epithelial cells, and capillary count upon tramadol use. Electron microscopy revealed various alterations in the ultrastructure, such as a considerable change in the mitochondrial shape and a decrease in the total number of them, whereas there was a considerable increase in the number of lysosomes. Moreover, an increase in the expression of inflammatory and apoptosis-related genes was detected in the CP following tramadol administration. According to their results, the authors suggested that tramadol induced neurotoxicity in the CNS through mechanisms involving apoptosis, inflammation, and oxidative stress.[85] It has been revealed that tramadol could substantially increase the expression profiling of genes encoding neurotoxicity in the prefrontal cortex and midbrain.[84]

Kamranian et al.'s (2018) research on the tramadol-treated animals' group showed a remarkable increase in the levels of lipid peroxidation, glutathione disulfide (GSSG), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) while depicting a dramatic decrease in the levels of GSH, SOD, glutathione peroxidase (GPx), glutathione reductase (GR), and mitochondrial quadruple complex enzymes in the hippocampus. In addition, based on their findings, authors suggested that the neurotoxicity effects of tramadol may be mediated by impacting the phosphoinositide 3-kinase (PI3K)/Akt/mTOR signaling pathways and its downstream inflammatory, apoptosis, and autophagy-related cascades.[86] (Table 1)

Table 1. *Experimental studies evaluating the effects of tramadolon the hippocampus*

Authors	Year	Dose	Administration route	Title	Ref
Kamranian et al.	2023	50 mg/kg	Gavage, daily for 14 days	Neuroprotective Potential of Trimetazidine Against Tramadol-Induced Neurotoxicity: Role of PI3K/Akt/mTOR Signaling Pathways	86
Gholami et al.	2023	25, 50, 75, 100, 150 mg/kg	i.p, daily for 21 days	Role of Apoptosis and Autophagy in Mediating Tramadol-Induced Neurodegeneration in the Rat Hippocampus	87
Khatami et al.	2022	In-vivo: 600 µm In-vitro: 50mg/kg	Gavage, daily for 3 weeks	Combined Molecular, Structural, and Memory Data Unravel the Destructive Effect of Tramadol on the Hippocampus	5
Imanpour et al.	2022	25 mg/kg	i.p, once time	The Effect of Orexin-2 and Endocannabinoid-1 Antagonists on Neuronal Activity of Hippocampal CA1 Pyramidal Neurons in Response to Tramadol in Rats	22
Hosseindoost et al.	2022	100-600 µg	Cell culture, added for 24, 48, and 72 h	Effect of Tramadol on Apoptosis and Synaptogenesis in Hippocampal Neurons: The Possible Role of µ-Opioid Receptor	77
Ahmadian-Moghadam et al.	2021	5, 10 mg/kg	i.p, daily for 14 days	Tramadol Treatment Induces Change in Phospho-Cyclic Adenosine Monophosphate Response Element-Binding Protein and Delta and Mu Opioid Receptors within Hippocampus and Amygdala Areas of Rat Brain	79
Hussein et al.	2020	40 mg/kg	Gavage, daily for 30 days	Tramadol Administration Induced Hippocampal Cell Apoptosis, Astrogliosis, and Microgliosis in Juvenile and Adult Male Mice	13
El-Kerdasy et al.	2020	42, 84, 168 mg/kg	Gavage, daily for 10 days	Histological and Immunohistochemical Study on the Effect of Tramadol Abuse on Cerebral Cortex and Hippocampus in Male Albino Rabbits	76
Niknamfar et al.	2019	1 µg/rat	Intra-CA1, once time	µ-Opioid Receptor in the CA1 Involved in Tramadol and Morphine Cross-State-Dependent Memory	35
Baghishani et al.	2018	50 mg/kg	Gavage, daily for 28 days	The Effects of Tramadol Administration on Hippocampal Cell Apoptosis, Learning and Memory in Adult Rats and Neuroprotective Effects of Crocin	75
Jafari-Sabet et al.	2018	2.5 and 5 mg/kg	i.p, once time	NMDA Receptors in the Dorsal Hippocampal Area are Involved in Tramadol State-Dependent Memory of Passive Avoidance Learning in Mice	60
Jafari-Sabet et al.	2018	0.5, 1 µg/mous	Intra-CA1, once time	Cross State-Dependency of Learning between Tramadol and MK-801 in the Mouse Dorsal Hippocampus: Involvement of Nitric Oxide (NO) Signaling Pathway	64
Mehdizadeh et al.	2017	20, 40, 80 mg/kg	i.p, daily for 30 days	Mitochondrial Impairments Contribute to Spatial Learning and Memory Dysfunction Induced by Chronic Tramadol Administration in Rat: Protective Effect of Physical Exercise	78
Ahmadi et al.	2017	-	-	Complex Partial Seizure and Hippocampus Atrophy Caused by Tramadol Abuse: A Case Study	48
Nafea et al.	2016	42, 84, 168 mg/kg	Gavage, daily for 3 days	A Study of the Neurotoxic Effects of Tramadol and Cannabis in Adolescent Male Albino Rats	15
Jafari-Sabet et al.	2016	0.5, 1 µg/mous	Intra-CA1, once time	Tramadol State-Dependent Memory: Involvement of Dorsal Hippocampal Muscarinic Acetylcholine Receptors	49
Yang et al.	2012	5 mg/kg	i.p, once time	Tramadol Pretreatment Enhances Ketamine-Induced Antidepressant Effects and Increases Mammalian Target of Rapamycin in Rat Hippocampus and Prefrontal Cortex	72
Bloms-Funke et al.	2011	57 and 100 mg/kg	i.p, once time	Tramadol Increases Extracellular Levels of Serotonin and Noradrenaline as Measured by In Vivo Microdialysis in the Ventral Hippocampus of Freely-Moving Rats	74
Yalcin et al.	2007	20 mg/kg	i.p, daily for 4 weeks	Antidepressant-Like Effect of Tramadol in the Unpredictable Chronic Mild Stress Procedure: Possible Involvement of the Noradrenergic System	73

4. Discussion

Based on the results of this study, it seems that tramadol, in addition to therapeutic uses, has many side effects on different parts of the body, particularly the nervous system, such as neuro-cognitive defects and movement and memory disorders.[3, 5] Therefore, further studies are essential to understand the precise mechanism of its impact on the nervous system.

Recent studies indicate that the hippocampus receives cholinergic projections from other parts of the CNS, which have critical roles in regulating various cognitive and executive functions.[88, 89] In addition, the anticholinergic properties of tramadol have been reported in the previous investigations.[90, 91] Therefore, researchers have proposed that the cognitive disorders that occur after utilizing tramadol could be attributed to its anticholinergic properties.[90] Moreover, it has been reported that tramadol could affect learning and memory by declining the levels of some intracellular mediators, including cAMP, cGMP, protein kinase A (PKA), and protein kinase C (PKC).[51, 75]

Existing evidence supports the idea that the balance between the antioxidant and oxidant systems is essential for normal neural cell functions. Also, there is ample evidence that increased production of ROS in pathological situations in the CNS negatively impacts the normal function of neuronal cells, which can lead to various neurological deficits.[92-94] In vitro and in vivo studies demonstrated that tramadol exposure could result in excessive ROS production (CAT, MDA, SOD) and a dramatic decrease in antioxidant elements (GSH) in the CNS, which has adverse effects on different components of neural cells, such as lipids, proteins, DNA, and organelles.[3, 5, 13, 95] An experimental study found that NO could lead to peroxynitrite production in the CNS of tramadol-treated animals, boosting oxidative stress.[96] Besides, other study results illustrated that tramadol could induce DNA damage and fragmentation by increasing 8-OHdG levels in rat's CNS.[97]

On the other hand, tramadol has a strong potential to disrupt normal mitochondrial functions. In this regard, several studies have shown that tramadol can induce MMP dysfunction, mitochondrial swelling, deterioration in the electron transfer chain (complex I, III, and IV), and increased of cytochrome c release in mitochondria.[21, 25, 84, 98-100] Also, it has been reported that rER, Golgi apparatus, and neuronal cytoskeleton could be affected by tramadol.

Recently, it has been suggested that oxidative stress may trigger inflammation by activating the redox-sensitive transcription factor nuclear factor-kappa B (NF-κB), Jun N-terminal kinase (JNK), and leading to the production of various inflammatory factors.[85, 87]

In an animal study, Mohamed et al. ascribed the inflammation observed in the rat brain due to tramadol to the activation of NF-κB. Their findings demonstrated that administering tramadol

orally for 8 weeks resulted in a substantial and dose-dependent elevation of the p65 subunit of NF-κB, along with increased levels of IL-6 and TNF-α mRNA in the rats' brains. Finally, they proposed that these elevations in the levels of oxidative and inflammatory factors following the tramadol administration could initiate and boost programmed cell death in the CNS.[96]

Furthermore, it is well-documented that inflammation plays a crucial role in the pathogenesis of neurodegenerative disorders.[101, 102] It is reported that due to pathophysiological conditions in the CNS, microglial cells become overly activated and release large amounts of inflammatory factors, which could ultimately have destructive effects on neural cells.[5, 102] Hence, some studies are carried out to examine the relationship between tramadol usage and inflammatory prosses in the hippocampus. According to the results of these studies, increased levels of neuroinflammatory factors, such as IL-1β, IL-6, IL-17, and TNF-α, have been reported after tramadol exposure.[3, 5, 96, 103] Prior studies have proposed that these inflammatory cytokines could cause direct neural cell death via activation of the TLR4 signaling pathway.[104]

The above factors can create the conditions for initiating destructive processes such as programmed cell death and autophagy. A large number of studies have shown a significant up-regulation in apoptotic (caspase-3, P53, Bax, Bid, TP53, P21, BBC3) and autophagic (light chain-3 [LC-3], autophagy-related gene-5 [ATG-5], ATG-12, lysosome-associated membrane protein-2 [Lamp-2], protein kinase B [AKT-1], microtubule-associated protein 1 light chain 3 beta [MAP1LC3B], Beclin-1 [BECN-1]) markers in the hippocampus following tramadol administration.[3, 5, 18, 105] Therefore, these processes can have many destructive effects on normal hippocampal formation, ultimately causing irreparable neural damage.

5. Conclusion

All in all, based on the results of previous studies, it is clear that tramadol can induce neural destruction in the hippocampus through probable processes, such as increased production of free radicals, dysfunction in some cell organelles, extreme production of inflammatory mediators, induction of apoptosis, and autophagy, ultimately leading to memory and cognitive disorders. Lastly, given the prevalence of illicit use of tramadol in addition to its therapeutic use in various communities, it is recommended that further research be conducted to determine the precise mechanism of the neurotoxicity of this medication and to prevent its adverse effects on the CNS.

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7. Authors' Contributions

Conceptualization: **S.V.N.**

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Formal analysis: **M.Sh.**

Investigation: **S.E.** and **M.V.N.**

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8. Conflict of Interests

The authors declared no conflict of interests.

9. Ethical Approval

Not Applicable.

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