

ADME and toxicity considerations for tramadol: from basic research to clinical implications

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REVIEW

ADME and toxicity considerations for tramadol: from basic research to clinical implications

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ABSTRACT

Introduction: Tramadol is widely used in chronic pain management for improving patients' quality of life and reducing trauma. Although it is listed in several medicinal guidelines, its use is controversial because of the conflicting results obtained in pharmacokinetic/pharmacodynamic studies. It is a multi-receptor targeting drug which acts as a μ_1 opioid receptor agonist, monoamine reuptake inhibitor, and inhibitor of ligand gated ion channels and some special protein-coupled receptors.

Areas covered: This review provides a comprehensive view on the pharmacokinetic, pharmacodynamic, and toxicity of tramadol with a deep look on its side effects, biochemical and pathological changes, and possible drug interactions. In addition, the main ways of tramadol poisoning management are described according to *in vivo* and clinical trial studies.

Expert opinion: Given the broad spectrum of targets, an increase in the cases of overdoses and toxicity, and probable interaction with other drugs, it is necessary to take another look at the pharmacology of tramadol. Regarding the adverse effects of tramadol on different tissues, especially the nervous system and liver tissue, more attentions to tramadol and its metabolites, their interaction with other drugs, and active agents seems critical. Seizure as the most cited effect of tramadol and its destructive effects on tissues would be alleviated by co-administration with drugs with antioxidant property.

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1. Introduction

Q4 25 Tramadol hydrochloride, a synthetic analgesic drug acting at the
central nervous system, is an opioid agonist that received Food
and Drug Administration (FDA) approve for managing pain in
1995 [1–3]. It is available in various formulations including drops,
extended release preparations, subcutaneous and intra venous
30 solutions [4]. Tramadol rapidly absorbs after oral administration
but its bioavailability is about 70% due to hepatic metabolism,
and it completely crosses the blood brain barrier. Its bioavail-
ability in buccal and nasal administration is much higher than
oral administration (~500% for nasal administration and ~200%
35 for buccal administration [5]). This opioid analgesic is widely
being used in reducing pain in cancer patients and some
acute pains like postoperative pains, back pain, rheumatoid
arthritis, and abdominal pain [6]. Some reports have also
revealed that it is efficient for reducing neuropathic pain [7]
40 and osteoarthritis related pains [8]. Besides, there is some evi-
dence of the use of this drug for premature ejaculation treat-
ment [9,10]. It was thought that tramadol has the lower risk of
overdose, constipation, and addiction than other opioids, but
more studies on its metabolites demonstrated several side
45 effects and phenotype differences in analgesia [11]. Tramadol
is used as a racemic mixture of two synergistic enantiomers. The
positive enantiomers (+) of tramadol can interact with both μ
and κ -opioid receptors as well as reducing spinal cord pain

transition by blocking norepinephrine (NE) and serotonin
(5-HT) uptake while the negative one (-) inhibits noradrenaline
50 uptake [12–14]. The tramadol affinity to μ opioid receptors (μ
OR) is much weaker than that of codeine (10 times) and mor-
phine (6000 times) [15]. It causes analgesia by interacting with
the nociceptive process just like tapentadol (a similar dual acting
55 analgesic). The only difference is that tapentadol acts without
active metabolite but tramadol is metabolized to
O-desmethytramadol (M_1) [16]. Tramadol administration for
chronic pain requires a deep understanding of its special phar-
macology, side effects, and drug-drug interactions. Unfortun-
ately, tramadol poisoning led to death due to respira-
60 tory depression, apnea, cardiopulmonary arrest, and seizure. Its
fetal level is significantly low (0.15–39 mg/L) when concurrently
taken with other drugs [17]. According to a cohort study by
Zeng et al. [18] higher tramadol mortality compared to
naproxen (23.5/1000 person-years vs 13.8/1000 person years),
65 diclofenac (36.2/1000 vs 19.2/1000), celcoxib (31.2/1000 vs 12.8/
1000) was demonstrated.

More understanding of the tramadol pharmacology, its side
effects and, clinical reports of tramadol overdoses and poisoning
would help us for reducing the side effects of this drug, elim-
inating the probable drug interactions and even helping us for
70 better drug formulation. The tramadol mechanism of action and
metabolism in the body is almost completely known, but this
drug can interact with several pathways, and understanding the

Article highlights

- The ADME (absorption, distribution, metabolism, excretion, and toxicity) of a tramadol are studied. Tramadol elimination half-life is about 5 h, and it is mainly excreted by kidneys and feces. O-desmethytramadol (M₁) is the main metabolite of tramadol. Its production rate and tramadol tolerance is directly related to the genetic variation in CYP450.
- Opioid receptors activation, monoamine transporters, G-protein-coupled receptors (GPCRs), muscarinic and nicotinic receptors, GABA receptors, NMDA receptors, and voltage dependent receptors are the main tramadol sites of action.
- The main side effects of tramadol in the terms of clinical and apparent side effects, biochemical, and pathological changes are discussed. Seizures, serotonin syndrome, headache, vertigo, hyperglycemia, hypotonicity, and acidosis are the general side effects of tramadol. Besides in some cases the low level of glucose and high CO₂ concentration in blood and respiratory depression in coordination with adverse effects at pathological levels to the lung, kidney, testicular tissues, and CNS are indicated.
- Poisoning management protocols for tramadol overdose are investigated. Naloxone, antioxidants, caffeine, ethimazole, morphine, acetaminophen, and benzodiazepines are mainly used together for both managing tramadol poisoning or as pain killer.
- Tramadol drug interaction as one of the main cause of tramadol related to side effects and deaths were explained by a few examples.

This box summarizes key points contained in the article.

connection between the wide sites of actions still require more studies. In addition, it is reported that tramadol has varied effects on different patients. It is because of differences in cytochrome P450 (CYP) activity and liver function in people. Furthermore, tramadol is metabolized to different metabolites in the body (M₁-M₅) and each of them has its own pharmacology. Although this drug is approved by FDA, recent studies have revealed several side effects related to tramadol abuses (especially high dose) and co-administration with other drugs. Here in this narrative review article, we made a deep look at absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of tramadol with emphasis on biochemical and pathological changes induced by this drug to different tissues. Literature research was performed in PubMed, Scopus, and ISI Web of Science using several the terms including tramadol ADMET, tramadol side effects, and tramadol abuses to identify literature for the discussed topic. However, research about tramadol is also faced with several limitations and there are several gaps in knowledge. One of the limitations of studying tramadol toxicity is designed a retrospective study and the inability to directly collect information from studies on humans. Also, limited information is available for a combination of tramadol and drugs. In addition, the tramadol studies are faced by debate about the legal status. Tramadol in several states of the USA is categorized as a schedule IV controlled substance. Finally, the authors believe that this study has limitations include restrict keyword search, and restricted search engines.

1.1. ADME of tramadol

1.2. Absorption

Oral, intravenous (IV), and subcutaneous are three ways of tramadol administration [19]. Recently, the transdermal way is

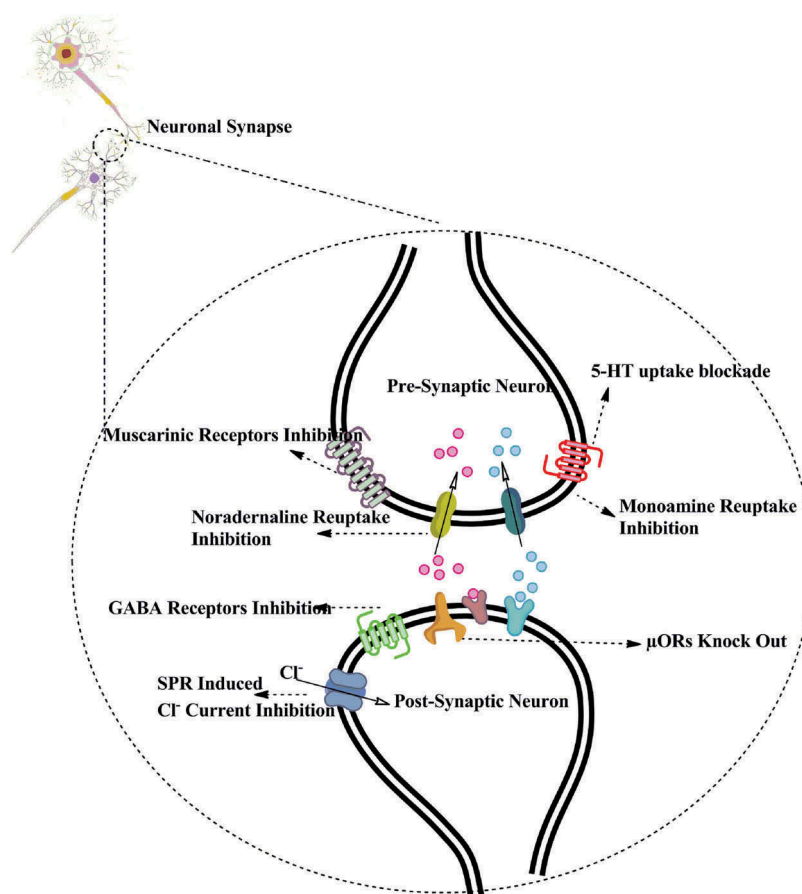
developed for facing the drawbacks of traditional methods [20]. Tramadol in its racemic form is used for oral administration. It is rapidly and completely absorbed in the intestine with 70% bioavailability [21]. The reduction in bioavailability is because of 20–30% first pass metabolism of tramadol [22]. The peak plasma concentration (C_{max}) of tramadol is reached after 1.2 h and 1.9 h for drops and capsules, respectively [21]. There is a linear correlation between the plasma concentration of tramadol and dose of administration (50–400 mg) [21,23]. The approximate bioavailability of this drug after 100 mg oral administration reached 300 µg/L which increased to 348 µg/L after four times of administration in a day. This enhanced bioavailability (up to ~100%) is probably due to the saturation of tramadol hepatic metabolism [22]. It is possible to reduce first-pass metabolism of tramadol by the administration of the drug through other ways. For instance, the administration of suppositories containing 100 mg tramadol led to higher bioavailability (77%) than oral administration. In addition, the C_{max} reduced to 294 µg/L after 3.3 h [24]. As it is expected, tramadol was completely absorbed after intramuscular injection. So that, after 0.75 h from an intramuscular injection, the C_{max} reached 166 µg/L. Transdermal delivery of tramadol (100 mg/g) in Pluronic Lecithin Organogel (PLO) and lipoderm resulted in 100% and 90% absorbance of the drug, respectively. Furthermore, the highest mean flux of tramadol reached 12.55 µg/cm²/h after 18 h for PLO and 14.21 µg/cm²/h after 10 h for lipoderm [25].

1.3. Distribution

After absorption of tramadol, it is extensively metabolized by O- and N-demethylation, followed by conjugation and urinary excretion. The elimination half-life (β elimination) of this drug is about 5 h and that of its main metabolite (M₁) is about 8 h. After oral administration of tramadol, it is mainly (~90%) excreted by kidneys and the remaining is excreted with feces. Tramadol excretion in patients with renal impairment is lower than healthy people [26]. The urine concentration of the tramadol is mainly one order of magnitude higher than tramadol concentration in heart blood, peripheral blood, liver, and kidney tissue [27]. M₁ is released into the blood and activates opioid receptors after entering into the central nervous system. Finally, it is inactivated by glucuronidation by the UGT2B7 enzyme of the liver [28]. In a study performed on zebrafish, nearly high concentrations of tramadol were observed in brain tissue after 0.5 and 1 h from drug administration. According to quantifying results of this study, the tramadol concentration increased up to 56.4 and 742.2 times in liver and gut tissues after passing only 0.5 h from tramadol administration. The concentration of tramadol in the eyes and muscles maintained at stable and low levels [29].

1.4. Metabolism

After the administration of tramadol, it is rapidly transported to the liver tissue. It first undergoes O- and N-demethylation and in the next step undergoes conjugations by cytochrome enzymes of the liver. The phase I metabolic reactions are the rate-limiting step of tramadol metabolism (Figure 1). Twelve metabolites are the results of tramadol liver metabolism and among them only O-desmethyl tramadol (M₁) and O, N-di-desmethyl tramadol (M₅)



Q8 **Figure 1.** Schematic representation of tramadol metabolism. Depicted based on [15,30–34].

are pharmacologically active. M_1 is the result of *O*-demethylation by CYP450 family 2 subfamily D, polypeptide 6 (CYP2D6) [4,15]. The levels of (-) M_1 is much higher than the other enantiomer ((+) M_1). Various metabolizer phenotypes (MP) are predictable by genetic variants in CYP2D6. People base on their CYP2D6 profile are grouped into poor (little CYP2D6 activity), intermediate (moderate CYP2D6 activity), extensive (the most common level of activity), and ultra (very high activity) metabolizers [30]. Regarding the diversity of CYP2D6 activity, the US FDA approved tramadol label has some information about tramadol metabolism by CYP2D6 [31]. As CYP2D6 is the main enzyme for tramadol metabolism and also it is easily saturated and is a high-affinity enzyme that represents low content of liver CYP (1–5%), toxicity at the recommended dose is possible in people with liver impairment [32]. CYP2B6 and CYP3A4 are responsible for *N*-demethylation of tramadol to *N*-desmethyl tramadol (M_2). Although CYP3A4 is involved in the metabolism of several drugs and is subjected to activation and inhibition by other drugs and metabolites, there are few studies about drug interactions between tramadol and other CYP3A4 substrates [33,34].

There are also reports on decrease tramadol clearance and valuable tramadol half-life increase in patients with renal impairment. This is due to the fact that tramadol metabolites and unchanged tramadol are mainly excreted in urine. M_1 and M_2 further degrade to next pharmacologically active metabolite *N*, *O*-didesmethyltramadol (M_5). The phase II metabolism of tramadol includes M_1 inactivation by glucuronidation, sulfation, and excretion into urine.

1.5. Excretion

More than 90% of tramadol is excreted by kidneys and little tramadol activity is observed in feces and saliva. In addition, it has been shown that after 30 minutes from intramuscular administration of tramadol (50 mg), the concentration of this drug in saliva and urine considerably increased [35]. After 5 h from tramadol administration, the saliva and urine concentrations of tramadol were 8 fold and 45 fold higher than its plasma concentrations, respectively. It has been revealed that the mean total clearance of tramadol from intravenous and oral administration of tramadol is 467 mL/min and 720 mL/min, respectively [4,36]. The average elimination half-life of tramadol is about 6 h [4,37]. In a case report of two lethal tramadol intoxication, high levels of tramadol and its metabolites (*O*-desmethyltramadol and *N*-desmethyltramadol) were found in urine samples. The ratio of *O*-desmethyltramadol/*N*-desmethyltramadol was evaluated as a factor for determining a delay between ingestion and death [24].

1.6. Mechanism of action

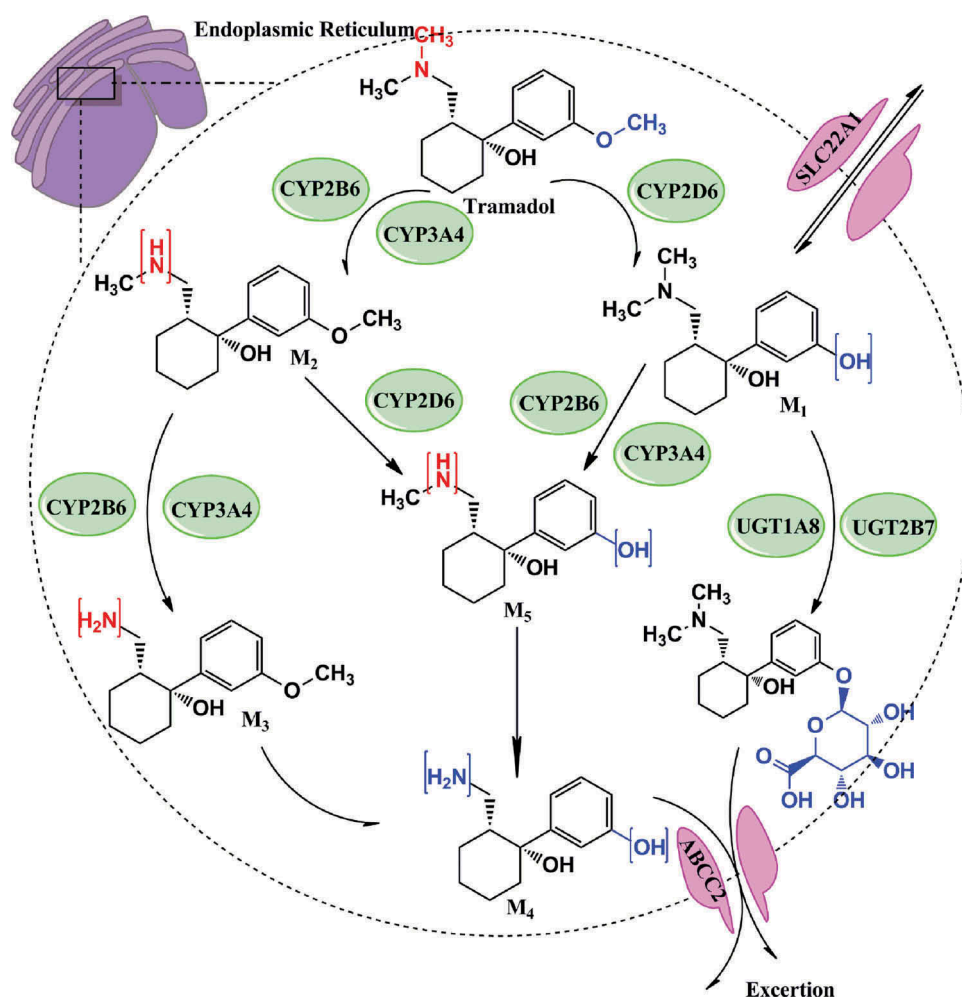
Opioid receptors (ORs) activation is the first and most understood mechanism of tramadol. It binds with moderate affinity to μ ORs and with weak affinity to σ and κ ORs. This affinity is much less than codeine and morphine affinity. *O*-desmethyl tramadol (M_1) is the main metabolite of tramadol which binds 300 times higher than tramadol to μ ORs. As naloxone

administration partially blocks the tramadol pain reduction, it is demonstrated that tramadol induces its effect through several different pathways (Figure 2) [15]. In another study performed on μ OR-knockout (μ OR-KO) mice, tramadol still inducing significant anti-nociceptive effects in homozygous μ OR-KO mice [38]. According to studies performed on tramadol co-administration with caffeine (nonselective A_1/A_2 receptor antagonist) and DPCPX (8-cyclopentyl-1, 3-dipropylxanthine; selective A_1 receptor antagonist), both caffeine and DPCPX inhibited tramadol's systemic anti-nociception. This means that tramadol induces its anti-nociception effects through A_1 receptors [39].

Monoamine transporters (like norepinephrine and serotonin) are the other sites of tramadol activity. It blocks pain transmitting in the central nervous system through monoamines reuptake inhibition [40]. Furthermore, those G-protein-coupled receptors (GPCRs) are targets of tramadol's analgesics and anesthetics effects [41–43]. GPCRs stimulation activates Ca^{2+} activated Cl^- channels by phospholipase C and then leads to the formation of inositol trisphosphate (IP₃) and diacylglycerol (DAG). The neuropeptide substance P receptor (SPR) is a GPCR which its role in anesthetic mechanisms attracts much attention after observing higher pain sensitivity of Sepiapterin reductase (SPR) knockout mice [44,45]. Studies

on *Xenopus* oocyte expression system have revealed that M_1 can induce an anti-nociceptive effect by inhibiting SPR induced Cl^- current [46,47].

The neurotransmitter 5-HTs belong to GPCRs (but not 5-HT_{3A} and 5-HT_{3B}) and involve several important physiological cells functions. The anti-nociceptive effects of tramadol are mediated by the serotonergic component, which is mediated by the 5-HT₂ receptor [48]. Tramadol and its M_1 metabolite can inhibit 5-HT-induced Cl^- currents and specific binding of [³H]-5-HT to 5-HT₂ C receptors [41,42]. 5-HT uptake blockade of tramadol and M_1 is probably the cause of serotonin transporter (SERT) and norepinephrine transporter (NET) inhibition by tramadol and its metabolites [49]. In addition, tramadol prevents the uptake of [³H]-NE into hypothalamic synaptosomes [50]. Tramadol also inhibited muscarinic receptors which have great roles in a wide range of CNS and autonomic nervous system functions. These receptors which are named because of their higher affinity to muscarine than nicotine are divided into five subtypes (M_1 - M_5) [51]. The muscarine-induced cyclic GMP accumulation in cultured bovine adrenal medullary cells and binding of [³H]-quinuclidinyl benzilate was suppressed by tramadol [52]. Furthermore, it has been indicated that tramadol specifically inhibited [³H]-QNB and acetylcholine (ACh)-induced currents binding and so inhibited



Q9 Figure 2. Schematic diagram of the sites of tramadol action. Depicted based on [38–43].

M₃ receptor function [53]. According to Nakamura et al. [54] the affinity of tramadol metabolite (M₁) is different from tramadol. They have revealed that M₁ can inhibit ACh-induced currents in oocytes expressing M₁ receptor while it had no significant effect on ACh-induced currents in cells expressing M₃ receptor. This little effect of M₁ on M₃ receptors explained the anticholinergic effect of tramadol [54]. The anticholinergic effect of tramadol was further investigated and it was shown that gastric acid secretion significantly reduced in response to tramadol [55]. The inhibitory effect of tramadol on M₁ and M₃ receptors suggesting that tramadol acts at cholinergic receptors [52]. ACh which is the activator of muscarinic and nicotinic receptors is the other site of tramadol action. According to Shiraishi et al. [56] tramadol suppressed catecholamine secretion induced by carbachol but had low effects on the induction of carbachol by veratridine. Beside the mentioned receptors, little effects of tramadol and its derivatives on several other receptors including GABA receptors, NMDA receptors, and voltage-dependent receptors have been reported [52–58]. Moreover, it has been reported the inhibitory effect of tramadol on vanilloid receptors. These receptors are shown to have a great role in the central integration of pain sensation [59].

1.7. Tramadol toxicity and side effects; from basic to clinical point of view

1.7.1. Toxicity

The fate of cells after exposure to toxic levels of tramadol has received considerable attentions. Nearly all drugs have some hepato-renal toxic effects due to the central role of these organs in drugs metabolism and excretion. Liver and kidney are the main sites of drugs metabolism and detoxification. According to a cross sectional study on chronic users of tramadol in Gaza strip, the significant higher activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were observed in tramadol abusers compared to control. In addition, there was significant increase in levels of alkaline phosphatase (ALP) and bilirubin in chronic tramadol abusers indicated that tramadol caused severe damage to liver and induced cellular toxicity. The elevated levels of BUN, uric acid and creatinine was also strongly demonstrated the kidney toxicity for the abuse of tramadol for more than 5 years [60]. In this regard, a significant increase in AST and ALT levels in rats receiving 40 and 80 mg/kg tramadol was demonstrated [61]. The necrosis and degeneration of epididymis cells were observed in rats treated with tramadol [62]. Besides, the hepatocyte and centrilobular necrosis as the main histopathological changes in liver of rats treated with tramadol were investigated [63,64]. Oxidative stress is probably involved in tramadol induced toxicity. So that, CYP2D6 polymorphisms could increase the hepatotoxicity through tramadol metabolites accumulation and consequently induction of oxidative stress [65,66]. The probable mechanism of tramadol to increase the level of ROS was explained by Mohamed et al. [67] A significant decrease in mitochondrial electron transport chain complex I, III, IV activity has been observed in rats treated with tramadol. Besides, the

probable role of dopaminergic system related to tramadol-induced oxidative stress has been demonstrated. Since, the inhibition of dopamin D₂ receptors results in high decrease in tramadol oxidative stress induction and mitochondrial dysfunction [68]. According to an in vivo study performed on rats, the chronic exposer (30–60 mg/kg for 8 weeks) to tramadol resulted in increased levels of apoptotic gene markers (p53 and BAX) and the pro inflammatory cytokines (IL-6 and TNF- α) [69]. Furthermore, the tramadol administration for two months resulted in enhanced levels of cerebral lipid peroxidation, nitric oxide, and reduction in GSH content in rats. It was indicated that tramadol activates the redox-sensitive transcription factor NF- κ B in a dose-dependant manner and subsequently leads to production of multiple inflammatory mediators. Also, it was shown that induced NF- κ B activation is associated with the increased levels of serum pro-inflammatory cytokines (TNF- α and IL-6), and cerebrum proapoptotic markers (p53 and Bax) expression [69]. The adverse effects of acute tramadol administration on rats brain, lung, and heart has been reported by Faria et al. [70] According to this study, the effective analgesic dose of tramadol (10–50 mg/kg) after 24 h caused an increase in serum AST/ALT ratio, LDH, and CK-MB activities and enhanced levels of brain samples lactate and heart and lung protein oxidation [70]. These findings strongly suggested that tramadol induces systemic inflammation [69,70]. The neurotoxic effects of tramadol has also been demonstrated by several studies. In this regard, impaired memory function by μ -opioid receptors activation [71], weight loss of rats brain [72], increased apoptosis and oxidative stress in rat cerebral cortex [73], and reduction of the expression of several genes in the hippocampus [74] are investigated as some of these toxic effects of tramadol. In addition, it is proved that actually and chronically using tramadol can impair memory. It has been indicated that tramadol abuses significantly elevate lipid peroxidation by reducing the antioxidant efficacy of brain tissues and thus led to memory impairment [75,76].

Furthermore, lung damage and neuronal degeneration in the rat brain are shown as toxic effects of tramadol [77,78]. In a study performed on Wistar rats, the biochemical changes, histopathological toxicity, and oxidative damages of single, intermediate and maximum recommended daily dose of tramadol was evaluated [70]. According to this study, tramadol increased protein oxidation in lung and heart tissues, and also degeneration of neurons and cell death were observed in brain tissue. Cellular tolerance induction is the other side effects of acute tramadol administration. Based on a study performed on Wistar rats for evaluating the putative role of tramadol on dopaminergic cellular content of the ventral tegmental area, high inhibition of neuronal firing up to 31% was reported as neuronal tolerance [79].

Red neurons (a histological marker of apoptosis) degeneration as a cause of cerebral disfunction were indicated in rats treated with 20–80 mg/kg tramadol for 10 days [80]. Recently, it is approved that tramadol causes genotoxicity in a dose-dependant manner and has low repair capability. The highest mutation induction and the lowest DNA repair were observed in mice treated with 75 mg/kg tramadol [81]. The activation of two stem cell progenitor markers (Nestin and Notch 1) in

response to acute tramadol toxicity has been evaluated in rats. This is probably due to the potent protective role of these two markers in response to cell injuries [82]. Necrosis were the predominant cause of cell death in response to tramadol exposure in human neuroblastoma SH-SY5Y cells. Furthermore, glucose uptake stimulation and ATP depletion due to the alterations of metabolic enzymes expression were demonstrated as the other toxic effects of tramadol [78].

1.7.2. Clinical side effects

Although tramadol has a low incidence of side effects, some cases of addiction and respiratory depression are reported. The accordance of respiratory depression by tramadol is very rare, but it is more likely to occur in persons with CYP2D6 duplication (high metabolizers) [83]. The rate of poisoning and deaths attributed to tramadol are rising and the reports on its abuses and induced toxicity are increasing [76,83]. It has been indicated that long-term use of tramadol leads to various side effects including vertigo, hyperglycemia, hypotonicity, seizures, serotonin syndrome, headache, acidosis, and some rare cases of ear infection, tinnitus, and vertigo [84–87]. Seizure is mainly the result of utilizing too much tramadol in people with kidney or liver problems, patients with epilepsy, and those who are addicted to alcohol [88,89]. The serotonin syndrome has also been reported as a side effect of tramadol. From 1997 to 2017, about 1000 cases of serotonin syndrome caused by tramadol were reported to FDA [90]. Multiple receptors including dopamine receptor, histamine receptor, and γ -amino butyric acid neurotransmission are involved in seizure induction by tramadol [83,91,92]. The concentration of norepinephrine in the brain increases in response to tramadol and leads to mild hypertension and tachycardia. A survey introduced gamma aminobutyric acid release inhibition as another mechanism of tramadol-induced seizure [91]. The chronic side effects of tramadol include dizziness, headache, dry mouth, premature heartbeats, hallucinations, visual disorders, and vertigo [93].

Cardiopulmonary arrest is reported as the rare side effect of ingesting 5 g tramadol [94]. In an experiment performed on 479 patients, ~75% of them showed ECG changes due to sodium channels blockage by tramadol [95]. Also, in another study performed on 114 cases with tramadol poisoning signs, the cardiopulmonary arrest was shown as the cause of death in patients who ingested more than 5000 mg tramadol [94]. Table 1 summarized some of the main adverse effects of tramadol on different tissues.

It is so important to be aware that the mentioned side effects have linear correlations with the concentrations of tramadol and its derivatives in patients' blood. For instance, according to an observational prospective study performed on 120 patients, a direct relationship between the rate of seizure and the tramadol and M_1 concentration was demonstrated [108]. Several studies have demonstrated the neurological disorders and sexual impairment upon long-term uses of tramadol. The increase in liver and kidney damages has mainly demonstrated in chronic tramadol administrations and little side effects are reported in acute uses [109,110].

1.7.3. Biochemical changes

The increase in liver enzymes, serum creatinine, and blood urea nitrogen is the result of kidney and liver damages due to chronic tramadol administration [111]. The animal studies have demonstrated in accordance with centrilobular congestion and focal necrosis of the rat's liver. Although no changes in malondialdehyde (MDA), AST, and blood urea were observed, tramadol hepatotoxicity was shown by observing a significant increase of ALT [109]. Furthermore, low level of plasma glucose due to reduction of liver gluconeogenesis in response to μ receptor activation by tramadol is reported in diabetic rats [112]. Also, the high level of CO_2 in blood samples in patients with tramadol overdose has been evaluated in several works as the sign of respiratory depression [110,113]. In retrospective study on 144 patients with acute tramadol poisoning, the laboratory findings including plasma electrolytes and blood gas were used for patients monitoring. The pure acute respiratory acidosis ($PCO_2 = 49.7 \pm 8.6$ mmHg, and $HCO_3^- = 24.1 \pm 3.8$ mEq/L) were observed in tramadol intoxicated patients [113]. The possibility of tramadol respiratory depression was highlighted after observing the dose-dependent (1–4 mg/kg) respiratory depression in cats [114]. The effects of tramadol on the hematological parameters are indicated in other studies. For instance, 28 days of tramadol administration (10–100 mg/kg) lowered white blood cell (WBC) count, red blood cell (RBC) count, and platelet (PLT), while enhanced the value of Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) [115]. It is also observed that Hematological alterations including RBC, WBC, MCV, packed cell volume (PCV), and hemoglobin (Hb) concentration reversed after tramadol withdrawal [116,117].

1.7.4. Pathological manifestations

Many studies evaluated the adverse effects of chronic tramadol administrations on different organs and tissues. The changes in internal organs upon chronic tramadol uses in 49 bodies investigated from the histopathological point of view [118]. The lung tissue analysis has shown enhancement of histopathological changes upon increasing the tramadol doses. The intra alveolar hemorrhage, alveolar collapse, inflammatory cell infiltration, and interstitial changes were indicated as the main histopathological changes in lung tissue in response to tramadol (25–50 mg/kg). Moreover, the altered cardiomyocytes and inflammatory cell infiltration were observed in heart tissue [70]. There are some reports on respiratory depression due to taking tramadol with alcohol, anesthetics, and sedative drugs [119]. The liver was shown as the tissue with the highest pathological changes due to tramadol uses. The incidence of atelectasis and edema in coordination with alveolar bleeding demonstrated as the major pathological finding in these bodies. Also, tramadol was found in stomach content, urine, blood, and gall bladder [118]. In an experimental study performed on sheep model, abnormal changes in liver and kidney samples including congestion and dilatation of veins, hepatocytes degeneration, swelling of tubular cells, dilation of intertubular blood vessels,

Table 1. Summary of some side effects and histopathological effects of tramadol on different tissues. AST: Aspartate transaminase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; MDA: Malondialdehyde; RA: Route of administration; TNF- α : Tumor necrosis factor α ; ALP: alkaline phosphatase.

Dose	RA	Duration	Species	Side effects	Major histopathological changes	Ref.
10, 25, 50 mg/kg	Intraperitoneal administration	24 h	Rat	Increase in AST/ALT ratio, LDH, and lactate levels	Protein oxidation in heart and lung tissues. Alterations of cells morphology, inflammatory cell infiltrations and cell death.	[70]
10, 50, 100, 200 mg/L	Exposing fish eggs with tramadol and naproxen	32 days	Fish	High mortality rate (50%) and elevated pigmentation in eggs exposed to 200 mg/kg tramadol	High skin mucous cells and gill lamellas deformation	[96]
4 mg/ml	Intra-articular administration	3 days	Human	Serotonin syndrome		[90]
100 mg	Oral administration	1 day	Rat	Induction of cellular tolerance		[79]
20 mg/kg	Intraperitoneal injection	1 day	Rat	Synergistic anti-nociceptive effect of tramadol and morphine co-administration		[151]
3.9–22.2 mg/kg	Intraperitoneal injection					
6.81–10 mg/kg	Intravenous administration		Human	High rate of hypoglycemia		[97]
1.25–10 mg/kg	Intraperitoneally injection	25 h	Dog	No anti-nociceptive effect was observed		[98]
40 mg/kg	Oral administration	30 days	Rat	Reduced mechanical hyperalgesia and edema Apoptosis in spermatogenic and ovarian cells	Ovarian failure and vanishment of follicles. Disorganization of seminiferous tubules.	[99] [100]
40 and 80 mg/kg	Oral administration	1 month	Rat	Increased level of ALT, LDH, and MDA. Reduction of serum glucose and cholesterol		[61]
22.5 mg/kg	Oral administration	9 weeks	Rat	Significant serum markers level enhancement (Urea, creatinine, TNF- α)		[101]
300 mg/kg	Oral administration	60 days	Rat	Significant liver specific enzymes enhancement (ALT, AST, ALP). The level of sex hormones and prolactin increased significantly		[102]
60 mg/kg	Subcutaneous injection	30 days	Rat	neurodegeneration		[103]
50 mg/kg	Oral administration	28 days	Rat	Dark neurons and apoptotic cells increased		[104]
40 mg/kg	Oral administration	6 weeks	Rat	High significant decrease Bcl-2 immunoreaction	Disorganized spermatogenic cells. Spermatogonia with vacuolated cytoplasm	[105]
12.5 mg/kg	Intraperitoneally injection		Rat			[106]
40 mg/kg	Oral administration	1 day	Rat	High tail flick latency	Congested blood vessels, degeneration of some hepatocytes, and vacuolation of hepatocytes in liver tissue.	[107]

and epithelial cells necrosis were observed. It was strongly approved that long-term use of tramadol has a direct correlation with hepatic and renal damages [119]. In addition, vacuolated hepatocytes and proliferated Kupfer cells with dark nucleas well as mild inflammation in liver tissue were observed in rats treated with tramadol [120]. The adrenal insufficiency by long term use of tramadol (80 mg/kg) in rats was approved by histopathological and immune-histochemical analysis [121]. Rats were treated with tramadol at a dose of 80 mg/kg for 3 months and then sacrificed and their adrenal glands were further examined. According to H & E images, loss of normal architecture in zona glomerulosa and zona fasciculate was indicated in animals treated with tramadol. The cells were ballooned and vacuolated with pyknotic nuclei. The blood capillaries of glands were congested as well. The mitochondria degeneration, lipid droplets, and dilation in cisternae were also observed in tramadol treated samples [121]. The effect of tramadol on hippocampal cells was evaluated by treating albino mice with tramadol. High numbers of dark granular neurons with deeply stained nuclei were indicated in histopathological sections. Also, irregularly arranged dark pyramidal cells were observed in the juvenile treated group [122].

The histopathological sections from testicular tissue of albino rats treated with tramadol showed distinct changes in the germinal epithelium [123]. Damages in seminiferous tubules, vacuolar degeneration, pyknotic nuclei in spermatogenic cells, and apoptotic giant cells were indicated at sites of injuries. This observation strongly suggested that tramadol administration has negative effects on male reproductive organs [123]. Such effects are probably due to the spermicidal effect of tramadol [124].

1.7.5. Drug interaction

Drug interaction is the main cause of tramadol poisoning and accidental deaths. This drug interacts with more than 761 drugs. Among them, the main drugs included antidepressants, tegretol, digoxin, MAO inhibitors, rifampin, quinidine, stress drugs, sedatives, alcohol, and blood thinners [55]. Co-administration of tramadol and serotonin inhibitor drugs like fluoxetine leads to serotonin syndrome. So that, tramadol blocked serotonin reuptake which in turn leads to an increase in plasma concentration of serotonin and serotonin release at high doses [86,125]. Furthermore, mydriasis and tachycardia as the main signs of serotonin syndrome are determined as the high risk factors for seizure [91,92]. Studies have reported a possible elevated risk of seizures especially when tramadol (even its therapeutic dosage) is administered with antidepressants, serotonin reuptake inhibitors, alcohol, and antipsychotics [126]. The tonic-clonic seizures as the general sign of neurotoxicity of tramadol are reported in several literatures and it is recommended to not administer tramadol to patients receiving SSRIs, antipsychotics, and TCAs [127,128]. Also, the seizure thresholds have a negative linear correlation with serotonin levels and intracerebroventricular methysergide (a serotonin receptor antagonist) [129]. Also, it appears that drug interaction has higher effects on enhancing poisoning symptoms than tramadol concentration. For instance, when tramadol is taken with special drugs like CNS

depressants, the occurrence of death would significantly enhance even at low tramadol dosages [130]. In addition, it is indicated that tramadol has a low fatality rate especially when it is used alone. Most of tramadol fatalities happened when this drug is used with the other central nervous system depressants [131,132]. In a study performed on 12 tramadol related deaths, the tramadol blood concentration was ranged from 0.03 to 22.59 mg/L and in all cases the cause of death was the co-ingestion and not tramadol alone [133]. In a case report of lethal tramadol intoxication by Michaud et al [134] . the tramadol blood concentration of 38.3 mg/L in association with alcohol and alprazolam at concentrations 1.29 g/kg and 0.21 mg/L have reported. The first study in which tramadol was the only cause of death was reported by Moore et al. [134]. The tramadol distribution in blood, liver, kidney and urine were 20 mg/L, 68.9 mg/kg, 37.5 mg/kg, and 110.2 mg/L, respectively.

1.8. Poisoning management and antidote

Tramadol overdose is in coordination with conscious impairment, seizures, respiratory depression, and serotonin syndrome [110,135]. Several drug combinations were considered for reducing tramadol side effects. Among them, managing tramadol-induced seizure by naloxone administration remains controversial. The exact mechanism of seizure induction by tramadol is still unknown, but some studies proposed inhibition of gamma-aminobutyric acid (GABA) receptors, and serotonin, and nitrous oxide pathways as the probable mechanisms of tramadol seizure induction [53,91]. According to one randomized clinical trial study, only one intravenous injection of 0.8 mg naloxone enhanced seizure effects of tramadol [136]. In contrast, another study has shown that 0.05 mg naloxone administration help tramadol management of post-seizure complaints [137]. In a study performed on rats, it was demonstrated that it is possible to efficiently reverse tramadol toxicity and seizures by diazepam/naloxone combination. It was shown that diazepam abolished seizure and naloxone improved ventilation. Furthermore, a meta-analysis study performed based on literatures from 1990 to 2017 revealed that naloxone therapy did not have a significant effect in reducing tramadol-induced seizure [138]. In a study performed on mice, it was shown that naloxone administration markedly instigates seizure in animals exposed to toxic levels of tramadol [91]. In a study performed using tramadol's enantiomers and metabolites, it was revealed that naloxone specifically attenuates the seizurogenic activity of – enantiomers (the non-opioid acting element) [4]. This observation in some way explains why naloxone can't attenuate seizurogenic effects of heroin and morphine. According to JRCSL (Joint Royal Colleges Service Liaison Committee, 2006) multiple seizures due to the naloxone administration which are probably due to the non-opioid content, should be treated with diazepam [139]. The possible mechanism by which naloxone reduces adverse effects of tramadol is that naloxone administration enhanced the density of opioid receptors and so it reduces the consumption of tramadol through blocking the stimulatory

G proteins [140,141]. Some studies investigated the efficacy of antioxidants in reducing the hepatotoxic effects of tramadol. Besides, the rate of reactive oxygen species (ROS) generation in seizure, epilepsy, and neuronal death is significantly high [142,143]. For instance, it has been indicated that vitamin E administration ameliorates tramadol hepatocellular damages (steatosis, apoptosis and, hydropic degeneration) and enhanced liver tissue antioxidant capacity [144]. Also, the potential effect of caffeine as an adenosine antagonist in reducing tramadol-induced seizure and oxidative damages were evaluated [145]. Oxidative stress biomarkers including MDA concentration were considered as degrees of toxicity due to the administration of drugs/chemicals in studies [146–148]. Co-administration of tramadol and caffeine significantly enhanced seizure threshold and decreased oxidative stress induction in brain mitochondria of mice (ROS formation, MDA level and protein carbonyl formation reduced from 30%, 35 μ M and 0.24 mM to 23%, 24 μ M, 0.12 mM, respectively) [145]. In addition, the accordance of seizure in patients who concomitantly take tramadol and benzodiazepines is less frequent. Besides, there is some evidence which indicates that ethanol can reduce the seizure threshold [64]. Several drug combinations have tested for evaluating their effect on tramadol analgesic efficacy and its related side effects. It is revealed that tramadol and ethimazole have antagonism effects at low concentrations but they showed synergy at the high doses [149]. This phenomenon was observed again about the association of tramadol and ascorbic acid [150]. Morphine is another drug with synergistic anti-nociceptive effects with tramadol. A low dose of tramadol is required for acute pain control when it is administered with morphine [151]. Also, tramadol combination with acetaminophen, metamizol and paracetamol has synergistic effects on acute pain management [152,153]. There are some reports on increasing the risk of tramadol intoxication when tramadol is co-ingested with benzodiazepines. The benzodiazepine levels in certain cases were in therapeutic ranges. This is probably due to the extensions of tramadol pharmacological activities and side effects [130,134]. A cohort study was performed for investigation of the likelihood of multiple seizures and the need for prophylaxis anticonvulsants therapy following tramadol overdose [154]. According to this study, the risk of multiple seizures in tramadol poisoning is very low and because of no morbidity in patients who due seize, the anticonvulsant therapy seems unnecessary [154]. The tramadol and benzodiazepine interactions are not well understood yet but some similarities between tramadol and buprenorphine including their affinity to μ -opioid receptors, modulation by naloxone and metabolism by CYP450 lead to some hypothesis about this interaction [155,156]. These similarities have shown that tramadol probably interacts with benzodiazepine like buprenorphine. The buprenorphine is a weak inhibitor of CYP3A and as benzodiazepines metabolism is mediated by CYP3A, it is demonstrated that buprenorphine and benzodiazepine have synergic effects on the CNS [157]. Although the CYP450 indications of tramadol and benzodiazepine have not been clearly understood, the high occurrence of tramadol/benzodiazepine fatalities is not purely random [130].

2. Conclusion

Tramadol is widely being used in reducing acute and chronic pains, dental pain, cancer pain, and also labor pain management. As tramadol can induce its effect through different signaling pathways, it is strongly probable that tramadol interacts with other drugs, especially the ones that share the same metabolic pathways. Therefore, it is critical to monitor its drug interactions carefully to avoid unforeseen side effects. Besides, several side effects have been reported for tramadol alone. Tramadol overdose causes drowsiness, headache, breath problem, low heart rate, coma, and even death. It also leads to addiction and its withdrawal is along with several typical and atypical withdrawal symptoms. Gastrointestinal pain, bone pain, diarrhea, nausea, anxiety, and rhinorrhea are the most common symptoms of tramadol withdrawal [158,159]. Sometimes tramadol withdrawal symptoms are similar to serotonin reuptake blockers. Besides, other atypical symptoms of tramadol withdrawal include panic attacks, confusion, delusion, paranoid thoughts, and severe anxiety [158,159]. Unfortunately, there are some reports on increasing the risk of suicide after tramadol use. Regarding the increasing number of overdoses, death, and wide toxicity of tramadol, and because of the mentioned side effects, designing new derivatives from tramadol with lower side effects and even better therapeutic effects, designing new alternative drugs, limiting its administration, and development of new formulations of tramadol is proposed as the feature challenges for solving tramadol problems.

Regarding the warns of scientists and manufacturers of tramadol about the risks of addiction and the adverse effects of tramadol co-administration with alcohol [160] and CNS depressant drugs, new insight into prescriptions and making more limitations on unauthorized sales are required. In addition, its abuses are significantly increasing and consequently the rates of deaths related to tramadol are increasing [160]. So that, designing new compositions of this drug with minimal side effects and higher specific action are needed for promoting this drug. It is possible to design the next generation of tramadol by considering the tramadol structure, similarity with other drugs, its site of actions and the mechanism of action and also its way of interaction with biological cascades and other drugs metabolic pathways. For instance, a new compound is developed by linking N-methyls to cyclohexane ring. It was shown that this compound does not bind to the μ -opioid receptor but had anti-nociceptive effects on mice and rats [161]. Support should be offered to patients suffering from seizures, depression, and addiction. Furthermore, it is important to address the risks of co-administration with CNS depressants, SSRIs, and alcohol.

3. Expert opinion

Detailed understanding of drugs ADMET would help scientists toward the development of new drug formulations with lower toxicity and high performance [162]. Tramadol is a pain-killer that has been used for alleviating different types of pains due to its diverse sites of action including μ OR,

A₁ receptors, monoamine transporters, SPR, 5-HTs, GABA receptors, NMDA receptors, voltage-dependent receptors, and vanilloid receptors. Regarding these wide ranges of receptors and the high chance of drug interaction, especially with drugs act with the same receptors, tramadol should be administered more carefully for avoiding the accordance of negative side effects. The interaction of tramadol with other drugs including antidepressants, tegretol, digoxin, MAO inhibitors, rifampin, quinidine, stress drugs, sedatives, alcohol, and blood thinners have been reported [51]. According to literatures, the risk of tramadol seizure induction significantly elevates when it co-administered with antidepressant drugs. Besides, enhanced lipid peroxidation in brain and neuronal degeneration has been shown as the main cause of tramadol brain impairment. Hyperglycemia, ear infection, cardiopulmonary arrest, hepatic system, and gastrointestinal system have been indicated as the rare effects of tramadol abuses. According to histopathological findings, alveolar collapse and hemorrhage in lung tissue, inflammatory cell infiltration in heart tissue, vacuolated and proliferated Kupfer cells in kidney tissue, edema, and alveolar bleeding in the liver are investigated as the main side effects of tramadol. There are several efforts on using different drug combinations for alleviating tramadol side effects. Among them, naloxone is the most studied drug with controversial results. Although some reports showed the low naloxone concentration administration (0.05 mg) alleviates tramadol seizure induction, the higher concentrations (0.8 mg) enhanced seizure accordance upon tramadol administration. Some studies demonstrated the positive effect of antioxidant administration on reducing the ROS induced tissue damages upon tramadol administration. Furthermore, there are few controversial reports on alcohol, caffeine, and benzodiazepines co-administration with tramadol.

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