

RETROSPECTIVE REVIEW OF TRAMADOL ABUSE

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Abstract: In the Egyptian community, tramadol abuse is considered an increasingly alarming phenomenon. The popularity and massive use of tramadol especially among Egyptian youth contribute to alleged usages for treatment of premature ejaculation also for the extension of orgasm and also increase sexual pleasure as it available in many online drug media and stores. However, this abuser life on a blade of a knife and is susceptible to another sexual dysfunction, memory and learning disorder and different metabolic disorders. This literature consults the phenomenon of tramadol abuse and its relation to sexual function, memory learning disorder and other metabolic disorders. It could be concluded that the increase in the prevalence of tramadol HCl dependency over other substances in the Egyptian community, calls for paying more attention from family, health and educational institutes. Tramadol may apply a very useful intervention for treating premature ejaculation, but its abuse bad effect may supply a possible demonstration for the unexplained delayed fertility as well as behavioral and the associated psychological changes. Also it could involve malfunction of the cerebral cortex which includes deficits in memory and the reduction in cognitive function which appeared in chronic abusers of tramadol.

Key words: tramadol; abuse; apoptosis; oxidative stress; cognitive

Introduction

Tramadol is an unscheduled atypical artificial opioid analgesic that prompts μ -opioid as well as monoamine receptor systems. This has been proved for marketing as safe analgesic in Germany since 1977 also in Sweden and the United States since 1995. Tramadol has a fundamental expansive scope of purposes in acute pain in case of postoperative trauma and also in chronic pain, as pain killer in case of cancer; however, it may also have rewarding/enhancing effects (1). It believed to lead to the possibility of restricted abuse in

contrast with another μ -opioid receptor agonists; however, lab information has suggested that it collaborate some of these other μ -opioid receptor agonists pharmacodynamic effects (2). Acute dosages of tramadol display a profile of outcomes comparable to those of opioid receptor agonists and may additionally impede abuse in populations (1). The rate of incidence for the tramadol abuse has been shown to be 69/1,000 individuals per year, and its annual rate of dependence is about 6.9/1,000 individuals (3). In the latest International Narcotics Control Board survey, of the 77 countries that responded on the issue of

tramadol abuse, it appears to be problematic for 32 countries (4).

The relevance between tramadol and sexual function is dialectical. However, there is a proof that males with premature ejaculation (PE) can profit by the off-label use of tramadol (5). These patients are prone to other problems such as sexual dysfunctions, including desire disorder or erectile dysfunction (6), diminished sexual confidence and overall satisfaction on sexual relationship (7), secondary hypogonadism (8), drug tolerance (6) and dependency (9) as well as risky sexual behaviours.

There are some definitions for the hazardous use of analgesics are proposed according to two recent consensus statements (10), the associated commentaries of Butler (11) and Sullivan (12).

A. Misuse: Opioid use in opposition to the guide or specific pattern of utilization, in spite of the absence or presence of unfavourable outcomes or harm.

B. Abuse: tramadol or opioid used for other purposes nonmedical, such as altering one's state of consciousness or euphoria.

C. Addiction: A continues using of drug in spite of harm, and craving or in ability to stop using the drug despite of its harmful effect.

Epidemiology of tramadol HCl abuse

According to the data from Intercontinental Marketing Services (IMS) Kilochem, global tramadol consumption rate of tramadol from 2006 to 2012 increased from 290 tons to 424 tons (a 42% increase) (13). The International Narcotics Control Board (INCB) included data at 2013 annual record on international increasing and development in the nonmedical use and abuse, illicit manufacture and illicit home and global spread of tramadol. This record (14) confirmed that thirty three countries, about 42% of those responding, reported abuse and nonmedical use of tramadol, typically supplying narrative data. Abuse of tramadol (two thirds of which is oral-dosage-form abuse) has shown to be growing in twelve of the nations (38% reporting such abuse) and was stable in another thirteen nations (about 42%).

Also, five nations announced suffering from widespread risk of tramadol abuse, while unlawful deal was listed in a restricted number of countries. Tramadol abuse was previously reported to be a dangerous issue in Togo, Mauritius, Iran, Libya, Egypt, Lebanon, Jordan, Gaza and Saudi Arabia (4). Epidemiological reviews and surveillance research have reported that it's consuming, abuse liability, dependency, diversion and overdoses have increased, which lead a number of nations to set tramadol under national control (1). These nations consist of both developing and developed countries, such as Mauritius, Egypt, Bahrain, Venezuela, Sweden, Iran, Saudi Arabia, Ukraine, Nigeria, Brazil, China, Japan, Australia, Lithuania, Jordan, the United States and the United Kingdom. Otherwise when compared with morphine, tramadol is said to have low dependence and abuse potentials worldwide (13), and these low potentials have been proven both in preclinical studies (15) and through scientific observation.

It has been proved that higher (200 and 400 mg), but not lower doses (50 and 100mg), also chronic oral tramadol intake, even in the therapeutic concentrations (100 to 300 ng/ml) for a dose of tramadol which always prescribed in treatment of mild to moderate pain, may lead to physical dependence, boost its effects and dose-dependent opioid like withdrawal symptoms (16). Therefore, tramadol was listed as a controlled substance in many countries in the world. Recently in Egypt, although tramadol is not manufactured in the country, it has become a major health problem, despite the Egyptian Ministry of Health having listed it as a controlled substance in October 2002. Also a national survey of Substance Use Disorders in Egypt proved that tramadol is one of the most frequently abused drugs (17).

In Egypt, 20% to 40% of adults (18) and 83% of adolescents (19) with substance use disorders were found use tramadol. Recently, according to data from the Anti-Addiction Fund's hotline, tramadol considered as the number one of tabulated drug abused about 40.7% of Egyptian drug users. This elevated level may be due to that people use tramadol to increase their performance and work power. So,

tramadol moved from schedule 3 to 1 by the Egyptian Ministry of Health as it highly addictive substances Tramadol was considered a very popular street drug in Cairo according to the information from the United Nations Office for Drugs and Crime (UNODC) that found 5 billion pills available for use in 2014 (20). On the other hand, International Narcotics and Law Enforcement Affairs reported that although Egypt was not a producer for this drug, there was highly significant consumption of the tramadol in the country, and this medicine has gone from being used as a pain reliever to being used as a recreational drug bought easily on the street and online store (21).

The main cause which make tramadol as popular drug and used massively, especially between the young and middle-aged people as it used in treatment of premature ejaculation and for orgasm extension and to increase sexual pleasure (22). Another study reported the prevalence of tramadol dependency among substance abusers, and also assesses the severity of addiction. About 43.94% of the patients used poly substances, while individual who used one substance were as follows: tramadol 30.30%, heroin 11.52%, sedatives and hypnotics 4.24%, alcohol 3.64%, cannabinoids 3.03%, nalbuphine 1.82%, and cocaine 1.52% (23).

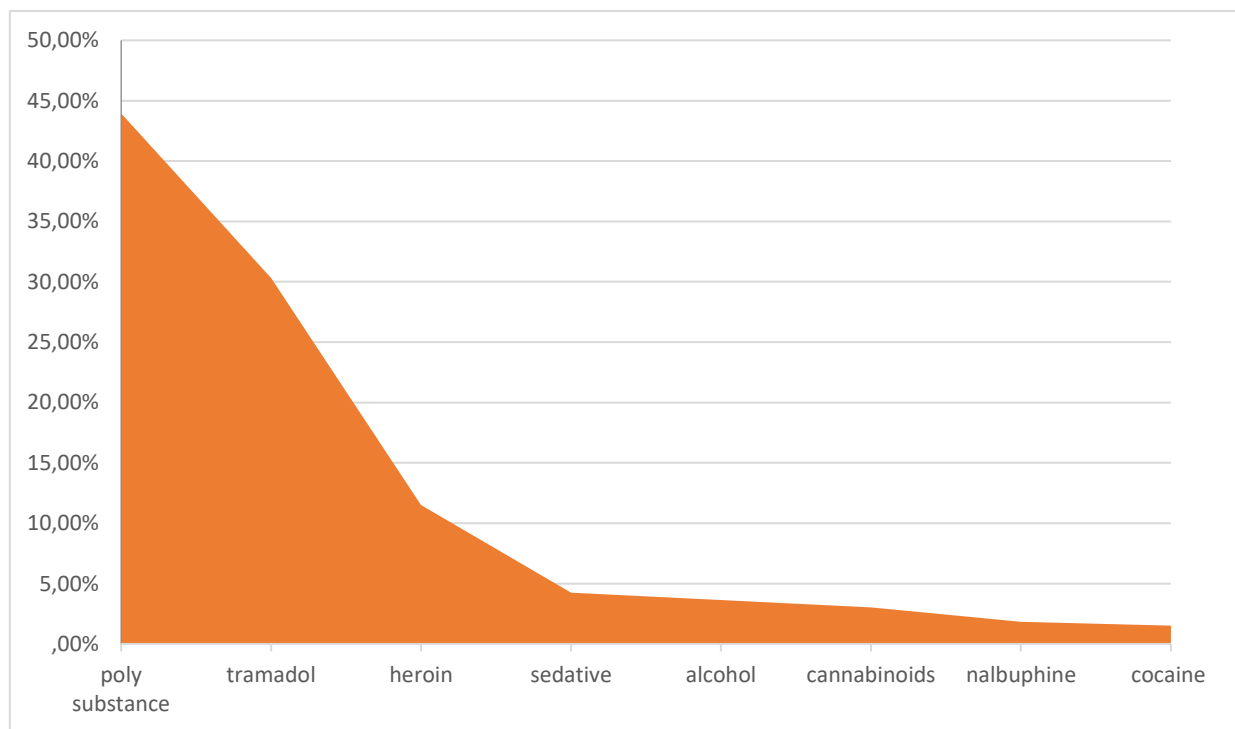


Figure 1: The prevalence of tramadol dependency among substance abusers, and assesses the severity of addiction in period of 12 months

Mohamed et al. (18) when analysed the percent of each drug among polysubstance, reported that the prevalence of cannabis within the polysubstance group was 83.4%, heroin 46.9%, sedatives and hypnotics 48.97%, tramadol 43.45%, and the prevalence of alcohol was 19.31% (23). Also, Hatata (24) reported that 61.9% of their participants used opiates, 18.5% used cannabis, 15.8% used sedatives, and 3.9% used alcohol. Moreover, Mohamed et al. (18) showed that opioids were the main

substance in 30% of their patient, especially tramadol. With regard to the gender, 80% were males and 20% were females. These findings make us wonder: the low number of female detected in the sample because of the low percentage of drug intake in between females or due to the difficulty and lack of availability of treatment options for women, or because of shame and stigma a woman, would encounter if she joins a substance dependence treatment program?

The prevalence of male over female reported in the WHO global survey (25), found that the estimated attributable burden due to illicit use of drugs is 0.8% among men and 2% among women. Also, Mohamed et al. (23) reported that 53% of the patients were single, 36% were married, 6% widowed and 5% divorced. Also 21.1% of the participants were married, 64.9% single, and 14% separated.

Abolmagd et al. (26) declared that 60% of the opioid users were from the middle socioeconomic level, while, 27% were from the high socioeconomic level, and 12.5% were from the high socioeconomic level. More than one-third of the substance abusers' fathers and almost half of their relatives were substance abusers. This indicates the effect of exposure to drug-related stimuli and the distorted models of fathers and relatives (27). The age of drug abuse onset was reported in the studies by Hafeiz (28), who found that, the age of onset was in the range of 21–32 years in 83% of the patients, which can prove the fact that most substance users are also within this age range.

Pharmaceutical form of tramadol

Hydrochloride salt is the form of marketing tramadol. Primarily route of administration is orally, although there are other available forms including intramuscular, subcutaneous, intranasal, intravenous and sublingual forms as well as rectal suppositories. Also we may find tramadol available in compound with acetaminophen and in extended-release or immediate-release formulations (29). After oral administration it is absorbed rapidly and completely, but its absolute bioavailability is only about 66–77% because of first-pass metabolism and bioavailability increases to almost 100% after an intramuscular administration or after multiple doses (30). The relative bio-availabilities in rats of the buccal and nasal-administration forms in comparison with the oral administration are about 183.4% and 504.8% respectively (31).

Pharmacokinetic of tramadol

Tramadol reach to peak levels after rectal administration in 3 hours, oral administration in 1-2 hours and intramuscular intake in 45

minutes. It has about 5–6 hours a terminal half-life (32). So tramadol need frequencies daily dosage about four to six times due to short half-life (33). While, it is extensively metabolized by demethylation, oxidation and conjugation in the liver (34) about 26 metabolites can be detected (fourteen phase I metabolites and twelve conjugates) (35) The O-desmethylation of tramadol metabolized to its main active metabolite, and then cytochrome P450 CYP2D6 catalyse O-desmethyltramadol (M1)(34) furthermore, CYP2B6 and CYP3A4 catalyse the N-desmethylation to N-desmethyltramadol (M2) (35) found only M1 and also N,O-didesmethyl tramadol (M5) are pharmacologically active Among all its metabolites (36). 90% of tramadol is primarily eliminated by the renal system and the remain part excreted with the stools (37). Tramadol excreted as the following percentages in 72-hour urine: 29% unchanged, 20% N,O-didesmethyl-tramadol and its conjugates, 20% O-desmethyl- tramadol and its conjugates, 17% N-desmethyl-tramadol, and the remainder percentage being other metabolites (38).

Mode of action of tramadol

Pharmaceutical formulation of it formed from racemic mixture of (+) tramadol and (-) tramadol, the tramadol antinociceptive effects included both nonopioid ingredients, which is serotonergic and noradrenergic components, also opioid ingredients which mainly act on the central nervous system (CNS) (39). Also other actions include influences on other several G protein coupled receptors (GPCRs), ion channel and transporters (40). On the other hand, clinical and preclinical studies demonstrated that the parent drug [(±)-tramadol, the two isomers] consider the only weak μ -opioid receptor which encoded by gene OPRM1 agonist (4,000-fold less than that of morphine), while the primarily responsible for μ -opioid activity of tramadol and is significantly more powerful than tramadol binding is metabolite M1 (400-fold higher affinity for the m-opioid receptor than that of tramadol, one-tenth that of morphine for the m-opioid receptor) (41). In case of analgesic effect (approximately one-third as potent as morphine

when each is administered orally) (42), while metabolites of tramadol M2, M3 and M4 have low affinity for the μ -opioid receptor of human (43). Otherwise another researches on acute administration reported minimal opioid-like effects. While chronic administration leading to opioid physical dependence and withdrawal on discontinuation (44). The dose of tramadol intake affect on the level of physical dependence. So large doses of tramadol can make a signal of abuse potential, also followed by high level of some prototypic measures like "drug liking" (1). CNS adaptations which result from accumulation of M1 likely are typical of other μ -opioid receptor agonists (45). The affinity of μ -opioid receptor only is not sufficient to interpret the analgesic action of tramadol. In addition to the action of tramadol on μ -opioid receptors, it act on noradrenergic and serotonergic systems may explain the involving of tramadol in the analgesic effect. Reuptake inhibition of serotonin by tramadol, with (+) tramadol about 4 times more effective than (-) tramadol, while M1 is about 10 times less potent than (-) tramadol (46). Likewise, in rat hypothalamic synaptosomes chronic and acute dose of tramadol holds the potential to block noradrenaline reuptake with (-) tramadol being approximately ten times more effective than (+) tramadol (47). There are other activities of tramadol including experimental evidence of inhibition of 5-hydroxytryptamine (5-HT) type 2C receptors (48), 5-nicotinic acetylcholine receptor (49), GABAA receptor (50), M1/M3 muscarinic receptor (51), N-methyl-D-aspartate (NMDA) receptor (50). Tramadol blocks catecholamine production at least partly by inhibiting functions of nicotinic acetylcholine receptors at clinically relevant concentrations in a manner independent of opioid receptors. One of the antinociceptive mechanisms exerted by tramadol is the inhibitory effects of tramadol on nicotinic acetylcholine receptor (52), and inhibition of transient receptor potential ankyrin 1 channel (TRPA1) (53). In the mouse formalin model there is a proof of involvement of cyclic guanosine monophosphate pathway, nitric oxide (NO) and ATP-sensitive K channels in antinociceptive function of tramadol (54).

Tramadol may make activation to the transient receptor potential vanilloid 1 channel (TRPV1) (55). Until now the significance of these later findings on the tramadol abuse is unknown; however, most of these receptors are involved in the regulation of sexual function.

Side effect of tramadol abuse

Oxidative stress can defined as in ability of antioxidant enzyme to neutralize reactive oxygen species (ROS) production. High level of reactive intermediates lead to damage in cell component which lead to production of secondary toxic compounds e.g., ketones and reactive aldehydes (56). Bajic et al. (57) concluded that chronic opioid administration may lead to oxidative stress which differs in relation to the age.

Previous *in vitro* studies, showed death of cell when exposed to opioid receptor agonists by apoptotic mechanisms (58). Also another studies in rats demonstrated that chronic opioid administration lead to serious changes in the proteins which involved in the apoptotic signalling pathway leading to induction of apoptosis (59). Otherwise at cellular level tramadol has toxic effects by increasing lipid peroxidation which may be used as a marker of damaged cell (60). In addition, Zhang et al. (61) showed that treatment with tramadol lead to increase of malonaldehyde (MDA) concentration, which suggests an increased level of lipid peroxidation. A reduction in the reduced glutathione level in the hepatocytes isolated from rat when incubated with different opioids concentration, a low level of reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase activities were detected.

Long term administration of tramadol have histological abnormalities on both testicular tissues and cerebral cortex with oxidative stress in these organs due to generation of lipid peroxidation and inhibition of gene expression of antioxidant enzymes leading to oxidative stress (62). Also, there is increase in apoptosis in the two organs which confirmed by increase in expression of Bax gene. Light and electron microscopic examination of both organs showed the toxic effects of tramadol through

two possible synergistic way, oxidative stress as well as apoptosis.

Nna et al. (63) conclude that chronic exposure to high doses of tramadol has negative effects on reproductive hormones in male rats, with poor reversibility following withdrawal. Apart from testicular oxidative stress previously reported by other researchers, also shown that up-regulation of prolactin a hormone known to correlate negatively with serum concentrations of testosterone, FSH and LH (hormones that support spermatogenesis) in males may play a major role in the etiology of reproductive deficit when chronically exposed to PDE5 inhibitors and opioids.

Lemarie and Grimm (64) Noticed the oxidative stress generation by administration of tramadol in the brain. Also, Mohamed et al (65). They explained effect of high dose of tramadol in mitochondrial electron transport chain (ETC) as it inhibited complexes I, III and IV. generation of reactive oxygen species due to Inhibition of complex III as an outcome of the intrinsic characteristics of electron transfer process to this complex from reduced ubiquinone. So, due to its high levels of oxygen consumption brain is mostly susceptible to oxidative stress (66). In mice chronic administration of tramadol leading to oxidative damage in tissue of brain, which followed by low level in brain non-enzymatic antioxidant, intracellular reduced glutathione level also glutathione peroxidase activity (67).

Furthermore, it has been reported that oxidative stress lead to lowering of enzyme activity and loss of function (68). Another study (69) demonstrated that the chronic effect of tramadol administration on insulin signaling pathway and neuronal glucose metabolism in the cerebral cortex which also leads to oxidative stress. Oxidative stress happened in different organ by administration of tramadol through induction of inflammatory reaction which reduced after withdrawal period (70). In rabbits after long term of chronic tramadol administration this inflammatory reaction confirmed previously to be a main factor for generation of oxidative stress by make changes in fatty acid composition of cell membrane leading to a decrease of its fluidity, which reduce formation

of pseudopodia and detect pathogens and foreign particles (71).

Recently, Ahmed and Kurkar (72) proved that administration of tramadol increased the testicular levels of lipid peroxidation and nitric oxide also significantly decreased the anti-oxidant enzymes activities in comparison with the control group as well as at level of immunohistochemical. They showed that tramadol in testicular tissues increased the endothelial nitric oxide synthase expression. Finally they concluded that the testicular function of adult male rats affected by tramadol treatment through generation of nitric oxide and oxidative stress stimulation by this drug.

Liu et al. (73) announced that the several effects due to long term use of tramadol and other opioid on neuronal structure (cytoskeleton) which considered as the signs for neuronal damage in the lymphocytes and mouse spleen, heart and lung. It may also stimulate the mRNA expression of pro-apoptotic receptors, through opioid receptor activation. Also large amount of apoptotic neurons in the hippocampus of these rats have been detected, and the expressions of the apoptosis related proteins bcl-2, Fas and caspase-3 presented with alteration. In comparison with control normal group, they noticed that marked increase in the expressions of Fas and caspase-3, while expression of Bcl-2 reduced significantly. They suggested that long term use of opioid drug lead to increase the apoptotic neurons through apoptosis related signaling pathway. This result was also confirmed previously by Atici et al. (74). They showed that chronic administration of tramadol and/or morphine with increase in the doses caused degeneration in red neuron and rat brain apoptosis, leading to cerebral dysfunction.

Recently, Sharifipour et al. (58) showed that chronic administration of tramadol in rats is a combined with a significant increase in the expression level of the pro-apoptotic Fas receptor, and upregulation in pro-apoptotic protein like caspase-3, which combined with decrease in the expression level of the anti-apoptotic oncoprotein Bcl-2.

Another study proved that under chronic tramadol administration the apoptotic index

showed highly significant increase in rat's testis tissue than that in the control group and decreased in rats under withdrawal (62). These results harmonized with another study using other opioids similar in action to tramadol (75). They confirmed that opioids have effects on secondary sex organs and spermatogenesis through a histological and pathological picture of apoptosis in rats receiving these drugs.

Another study showed neurobehavioral, neuropathological and neurochemical brain changes after co administration of nicotine and tramadol to male albino rats. Nicotine magnify oxidative stress produced by chronic administration of tramadol as proved by increase in nitric oxide and thiobarbituric acid reactive substances followed to the high amount of nitric oxide synthases. Also reduction of non-protein sulfhydryls was detected (76). Aggravation of oxidative damage by tramadol followed by increase level of nuclear factor kappa B (NfκB) and also setup in the proinflammatory cytokine as tumor necrosis factor α (TNF α) and appear of apoptosis obviously by the increased caspase-3 immunoreactivity as well as increased tyrosine hydroxylase in midbrain a comhgvrlned by high of butyryl cholinesterase and acetyl cholinesterase level. These results provide evidence that such combination aggravated neurotoxic effects and elicited negative effects regarding learning and memory.

Lees (77) stated that (Na⁺ & K⁺-ATPase) enzyme supports the cells ionic homeostasis also support keeping of neuronal resting membrane potentials and production of neural impulses. Subsequently, impair the activity of this enzyme will lead to cellular depolarization followed by brain dysfunction. As well as, on the same lines, the significant reduction in the activities of brain Na⁺ & K⁺-ATPase enzyme was reported (78) administration of either morphine or tramadol at chronic dose level, leading to cell injury and death of neurons, followed by brain disorders, which is in the same line with previous studies (79). Another illustration is that the tramadol may labor their inhibitory actions by its direct effect on the brain enzyme protein itself. According to previous findings (78), the considerable change

in activity of Na⁺, K⁺-ATPase enzyme, which is consider an integral membrane enzyme that enhance the process of Na⁺ and K⁺ ions transportation against concentration gradients, which lead to changes in Na⁺ and K⁺ concentrations as Na⁺ and K⁺ ions have a very important role in body functions which include nerve signals and impulse transmission, various chemical reactions and fluid balance. Impair the function of Na⁺ & K⁺-ATPase enzyme in brain considered the main cause of various types of neurological disorders, which is in the same line with another study done by Horvat et al. (80), who proved that abnormal excessive release of certain neurotransmitters amount caused by depolarization of cell membrane.

Another study done by Elwy and Tabl (78) demonstrated the potent effects of tramadol abuse on the activities of Acetylcholine esterase enzyme The actions of the drug were contributed to reduce in cognitive function and the memory deficits in chronic users. As a consequence, this bad effect of tramadol considered as a signs for drug-induced neurotoxicity.

Effect of chronic administration of tramadol in rat hippocampus may disturb learning and memory. It has also bad neurotoxicity effects on inducing dark neurons DN_s formation and apoptosis (81). In addition, this result harmonized with another study which reported that tramadol deteriorates memory when administered in acute or chronic dose. Also, tramadol when was administrated in a single dose reported more damaging effect on the memory when compared with multiple doses. This interpreted by the inhibitory effectiveness of tramadol on different type of neurotransmitters and numerous receptors as N-methyl D-aspartate, muscarinic, AMPA. It affect on some second messenger like cGMP and cAMP or it has stimulatory effect on the gama amino butyric acid ,opioid, serotonin or dopamine receptors in the brain (82).

Abuse as well as acute overdose of tramadol has led to reported cases of hepatotoxicity and even death in humans (83). Many studies in animals have proved hepatotoxicity with alteration on levels of liver function biomarkers (84) and histological damage was also reported

(85). Although, oxidative stress may be involved in tramadol induced hepatotoxicity due to decrease in the antioxidant enzymes and lipid peroxidation observed in treated animals (86).

On the other hand, another study showed biochemical and histopathological changes in kidney and liver of rats due to chronic usage of tramadol. Aspartate, Serum aspartate amino transferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), and creatinin levels were significantly increased when compared with the control group. Also Serum LDH level was significantly increased. The mean malondialdehyde (MDA) level was significantly decreased. Examination with Light microscope showed severe centrolobular congestion as well as focal necrosis in the liver of rat administrated chronic dose of tramadol. The main histopathological changes observed in group treated by tramadol were vacuolization in tubular cells. They also illustrated the hazard of exacerbated lipid peroxidation, renal and hepatic destruction due to long term use of tramadol (87).

While, activities of Super Oxide Dismutase (SOD), glutathione (GSH) and catalase (CAT) were significantly reduce. Whilst an increased in the level of MDA in both liver and kidney tissue of chronic administrated tramadol rats was observed. Long term tramadol treatment induced histopathological changes in both tissues. These alterations were manifested by severe hydropic degeneration, with congested central veins of the liver and degenerated renal tubules and atrophied glomerulus. While at level of mRNA expression of proapoptotic marker as expression of Bax gene showed a highly significant increase and the antiapoptotic Bcl-2 expression decreased significantly. Therefore, tramadol have harmful effect at the cellular level and can induce apoptotic changes in these tissues (88)

On the other hand, Boshra (89) evaluated the effect of tramadol administration on the adult female rats bone. In chronic administration; tramadol had the lowest osteoporotic effect in comparison with other opioid. Hence, use of tramadol is safe in case of treating patients who

suffering from chronic pain specially associated with osteoporosis.

Opioid drugs have a profound effect on sleep. Opioid receptors present in the ventrolateral preoptic nucleus, which is the same nucleus that is involved in regulation of sleep and considered as a sleep promoting area. It has been suggested that opioid peptides are involved in the induction of the sleep. They also cause excessive daytime sleepiness and fatigue (90). In the case of use of tramadol and opioid analgesics, despite the particular amelioration in sleep reported by patients on low doses of opioids, long-term administration of opioids affect sleep physiology. Opioids tend to disrupt the quality and quantity of sleep (91). Opioids can cause irregular and slow respiration leading to hypercapnia which followed by hypoxia (92). Chronic use of tramadol has also been accompanied with both obstructive and central sleep apnea through relaxation of the throat muscles in 30% to 90% of abusers (93). Tramadol is suspected to affect sleep. In addition to its effect on opiate receptors, it inhibits the reuptake of serotonin and norepinephrine, which in turn enhance its stimulating properties, that may further affect sleep quality (94).

In 2007, illustration in Ireland showed that the different critical complications like severe liver failure also heart failure, which may led to the death of patients, have been demonstrated. Only two cases presented that the only reason for his death was announced net tramadol. In autopsy examination of the cadaver post-mortem noticed changes as alveolar haemorrhage (bleeding in the air sacs), acute necrosis in renal tubular and ischemia in liver (95).

Tjäderborn (96) studied unintentional poisoning and fatal tramadol among Swedish forensic autopsy cases between 1995-2005. Seventeen patients (11 men and 6 women) have been recognized. For these cases, the range of age was from 18 to 78 years with average age 44 years and the median tramadol concentration was 2 µg/g.

In another study, ten cases (59%) of numerous drug poisoning was considered as the cause of death. while, in seven cases, tramadol

was the only drug which present in toxic concentrations. Also a History of different substance abuse in 14 cases (82%) was reported and recent history of tramadol abuse detected in eight patients (47% women). This demonstration proved that tramadol fatal poisoning may have occurred unintentionally and people with a history of substance abuse may be life on knife edges and high risk, so when history of tramadol use in these patients, they need caution (97). Another study in mice, revealed that tramadol acute intoxication when compared with control group, showed pathological changes in the livers and inflammatory cells infiltration into alveolar, haemorrhage and pulmonary oedema congestion in 95% of the mice. But in the control group which received normal saline they noticed normal histological liver tissue (98).

According to fatal and risky symptoms appear after chronic administration of tramadol such as prolonged hospitalization for poisoning, cardiac arrest and loss of consciousness. Type of subsequent complications, required attention to tramadol use poisoning. As in the case of death for poisoning was seen within tramadol user, mortality was most prevalent in young men. More attention and care for the elderly, in terms of risk of aspiration, which indicates a lack of defence mechanisms of prevention of these complications. Therefore prevention of intractable use of tramadol and care after poisoning with it is clear. After the death, the most important diagnostic aid will be biological samples with drug poisoning, first stomach contents and then urine samples (99).

Conclusion

The increase in the prevalence of tramadol HCl dependency over the other substances in the Egyptian community calls for pay more attention from family, health and educational institutes. Tramadol may apply a very useful interference in treatment of premature ejaculation, but its abuse risky effect may supply a possible demonstration for the unexplained delayed fertility as well as behavioral and the associated psychological changes. Also it could involve malfunction of cerebral cortex which includes memory disturbance and decrease in

cognitive function. Moreover, the appearance of various metabolic disorders in different body tissues in chronic users was detected.

Conflict of interest

The authors declare no conflict of interest.

References

1. Babalonis S, Lofwall MR, Nuzzo PA, Siegel AJ, Walsh SL. Abuse liability and reinforcing efficacy of oral tramadol in humans. *Drug Alcohol Depend.* 2013; 129: 116–24.
2. Stoops WW, Lofwall MR, Nuzzo PA, Craig LB, Siegel AJ, Walsh SL. Pharmacodynamic profile of tramadol in humans: Influence of naltrexone pretreatment. *Psychopharmacology (Berl).* 2012; 223: 427–38.
3. Knisely JS, Campbell ED, Dawson KS, Schnoll SH. Tramadol post-marketing surveillance in health care professionals. *Drug Alcohol Depend.* 2002; 68: 15–22.
4. Abdel-Hamid IA, Andersson K-E, Waldinger MD, Anis TH. Tramadol abuse and sexual function. *SMR.* 2016; 4: 235–46.
5. Martyn-St James M, Cooper K, Kaltenthaler E, Dickinson K, Cantrell A, Wylie K, Frodsham L, Hood C. Tramadol for premature ejaculation: A systematic review and meta-analysis. *BMC Urol.* 2015; 15: 6.
6. Alghobary M, El-Bayoumy Y, Mostafa Y, Mahmoud EHM, Amr M. Evaluation of tramadol on demand vs. Daily paroxetine as a long-term treatment of lifelong premature ejaculation. *JSM.* 2010; 7: 2860–7.
7. El-Hadidy MA, El-Gilany A-H. Physical and sexual well-being during and after tramadol dependence. *MECPsych.* 2014; 21: 148-51.
8. Abdellatif R, Elgamal D, Mohamed E. Effects of chronic tramadol administration on testicular tissue in rats: An experimental study. *Andrologia.* 2015; 47: 674–9.
9. Al-Fannah J, Bourin M, Brands B, Buitrago R, Dodoo A, Elliott S, Mahlangu G, Noveria P, Pennings E, Razaghi E. Who expert committee on drug dependence thirty-sixth report introduction. Who expert committee on drug dependence: thirty-sixth report. 2015; 991: 1–50.
10. Smith SM, Dart RC, Katz NP, Paillard F, Adams EH, Comer SD, Degroot A, Edwards RR, Haddox JD, Jaffe JH. Classification and definition of misuse, abuse, and related events in clinical trials: Action systematic review and recommendations. *PAIN®.* 2013; 154: 2287–96.

11. Butler S. The impact factor or impact strikes again! *Pain*. 2013; 154: 2243–4.
12. Sullivan M. Clarifying opioid misuse and abuse. *Pain*. 2013; 154: 2239–40.
13. Radbruch L, Glaeske G, Grond S, Münchberg F, Scherbaum N, Storz E, Tholen K, Zagermann-Muncke P, Zieglgänsberger W, Hoffmann-Menzel H. Topical review on the abuse and misuse potential of tramadol and tilidine in Germany. *Subst Abus*. 2013; 34: 313–20.
14. Sairam Atluri G, Manchikanti L. Assessment of the trends in medical use and misuse of opioid analgesics from 2004 to 2011. *ASIPP*. 2014; 17: E119-E28.
15. O'Connor EC, Mead AN. Tramadol acts as a weak reinforcer in the rat self-administration model, consistent with its low abuse liability in humans. *Pharmacol., Biochem. Behav.* 2010; 96: 279–86.
16. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an atypical opioid analgesic. *J Pharmacol Exp Ther*. 1992; 260: 275–85.
17. Hamdi E, Gawad T, Khoweiled A, Sidrak AE, Amer D, Mamdouh R, Fathi H, Loza N. Lifetime prevalence of alcohol and substance use in Egypt: A community survey. *Subst Abus*. 2013; 34: 97–104.
18. Mohamed NR, Hammad SA, El Hamrawy LG, Rajab AZ, El Bahy MS, Soltan MR. Dual diagnosis and psychosocial correlates in substance abuse in Menoufia, Egypt. *Menoufia Med J*. 2013; 26: 114.
19. Bassiony MM, Salah El-Deen GM, Yousef U, Raya Y, Abdel-Ghani MM, El-Gohari H, Atwa SA. Adolescent tramadol use and abuse in Egypt. *The Am J Drug Abuse*. 2015; 41: 206–11.
20. Asaad T, Barakat D, Elwasify M, Aziz KA, Elwasify M, El-Gabry DA. Screening for obstructive sleep apnoea in patients with treatment-resistant depression: A case-control study. *MECPsych*. 2018; 25: 57–63.
21. Brownfield WR. International narcotics control strategy report: Volume i: Drug and chemical control: DIANE Publishing; 2011.
22. Salem EA, Delk JR, Wilson SK, Bissada NK, Hellstrom WJ, Cleves MA. 1043: Tramadol hcl has promise in on demand use to treat premature ejaculation. *JU*. 2007; 177: 345.
23. Mohamed NR, El Hamrawy LG, Shalaby AS, El Bahy MS, Allah MMA. An epidemiological study of tramadol hcl dependence in an outpatient addiction clinic at Heliopolis psychiatric hospital. *Menoufia Med J*. 2015; 28: 591.
24. Hatata HA, Khalil A, Asaad T. Dual diagnosis in substance use disorders. An Egyptian study MD thesis Egypt: Faculty of Medicine, Ain Shams University. 2004.
25. Organization WH. Neuroscience of psychoactive substance use and dependence: WHO; 2004.
26. Abolmagd S, Nasr A, Erfan S, Mamdouh R, El-Lawendi M. A group of Egyptian addicts: Users view for addiction causes and its impact on self and family. *EJ P*. 2004; 23: 145–58.
27. Okasha A. Long term strategy of addressing narcotics—an Arab-Egyptian perspective. Read at the Royal College of Psychiatrists in Jordan, Amman. 2000.
28. Hafeiz H. Socio-demographic correlates and pattern of drug abuse in eastern Saudi Arabia. *Drug Alcohol Depend*. 1995; 38: 255–9.
29. Kaye AD. Tramadol, pharmacology, side effects, and serotonin syndrome: A review. *ASIPP*. 2015; 18: 395–400.
30. Lintz W, Barth H, Becker R, Frankus E, Schmidt-Böthelt E. Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 2nd communication: Drops with ethanol. *Arzneimittelforschung*. 1998; 48: 436–45.
31. Zhang H, Zhao Y, Wang X, Zhang Q. Bioavailability of tramadol hydrochloride after administration via different routes in rats. *Biopharm Drug Dispos*. 2014; 35: 525–31.
32. Raber M, Schulz H-U, Schürer M, Krupp S, Momberger H. Pharmacokinetic properties of tramadol sustained release capsules. *Arzneimittelforschung*. 1999; 49: 594–8.
33. Cnota PJ, Nowak H, Tagarro I, Erb K, Schürer M, Schulz H-U, Maus J. Tramadol sr formulations. *Clin Drug Investig*. 2005; 25: 435–43.
34. Stamer U, Musshoff F, Kobilyay M, Madea B, Hoefl A, Stuber F. Concentrations of tramadol and o-desmethyltramadol enantiomers in different CYP2D6 genotypes. *Clin Pharmacol Ther*. 2007; 82: 41–7.
35. Wu W, McKown L, Liao S. Metabolism of the analgesic drug Ultram® (tramadol hydrochloride) in humans: API-MS and MS/MS characterization of metabolites. *Xenobiotica*. 2002; 32: 411–25.
36. Gong L, Stamer UM, Tzvetkov MV, Altman RB, Klein TE. PharmGKB summary: Tramadol pathway. *Pharmacogenet Genomics*. 2014; 24: 374.

37. Lintz W, Erlacin S, Frankus E, Uragg H. Biotransformation of tramadol in man and animal (author's transl). *Arzneimittelforschung*. 1981; 31: 1932–43.
38. Lintz W, Barth H, Osterloh G, Schmidt-Böthelt E. Bioavailability of enteral tramadol formulations. 1st communication: Capsules. *Arzneimittelforschung*. 1986; 36: 1278–83.
39. Vazzana M, Andreani T, Fangueiro J, Faggio C, Silva C, Santini A, Garcia M, Silva A, Souto E. Tramadol hydrochloride: Pharmacokinetics, pharmacodynamics, adverse side effects, co-administration of drugs and new drug delivery systems. *Biomed Pharmacother*. 2015; 70: 234–8.
40. Minami K, Ogata J, Uezono Y. What is the main mechanism of tramadol? *Naunyn-Schmiedeberg's archives of pharmacology*. 2015; 388: 999–1007.
41. Raffa RB. Basic pharmacology relevant to drug abuse assessment: Tramadol as example. *J Clin Pharm Ther*. 2008; 33: 101–8.
42. Epstein DH, Preston KL, Jasinski DR. Abuse liability, behavioral pharmacology, and physical-dependence potential of opioids in humans and laboratory animals: Lessons from tramadol. *Biol Psychol*. 2006; 73: 90–9.
43. Smith HS, editor *Opioid metabolism*. Mayo Clin Proc; 2009: Elsevier.
44. Camí J, Lamas X, Farre M. Acute effects of tramadol in methadone-maintained volunteers. *Drugs*. 1994; 47: 39–43.
45. Duke AN, Bigelow GE, Lanier RK, Strain EC. Discriminative stimulus effects of tramadol in humans. *J Pharmacol Exp Ther*. 2011; 338: 255–62.
46. Gobbi M, Mennini T. Release studies with rat brain cortical synaptosomes indicate that tramadol is a 5-hydroxytryptamine uptake blocker and not a 5-hydroxytryptamine releaser. *Eur J Pharmacol*. 1999; 370: 23–6.
47. Franceschini D, Lipartiti M, Giusti P. Effect of acute and chronic tramadol on [3h]-norepinephrine-uptake in rat cortical synaptosomes. *Prog Neuropsychopharmacol Biol Psychiatry*. 1999; 23: 485–96.
48. Ogata J, Minami K, Uezono Y, Okamoto T, Shiraishi M, Shigematsu A, Ueta Y. The inhibitory effects of tramadol on 5-hydroxytryptamine type 2c receptors expressed in xenopus oocytes. *Anesth Analg*. 2004; 98: 1401–6.
49. Shiraishi M, Minami K, Uezono Y, Yanagihara N, Shigematsu A, Shibuya I. Inhibitory effects of tramadol on nicotinic acetylcholine receptors in adrenal chromaffin cells and in xenopus oocytes expressing $\alpha 7$ receptors. *Br J Pharmacol*. 2002; 136: 207–16.
50. Hara K, Minami K, Sata T. The effects of tramadol and its metabolite on glycine, γ -aminobutyric acid, and n-methyl-d-aspartate receptors expressed in xenopus oocytes. *Anesth Analg*. 2005; 100: 1400–5.
51. Nakamura M, Minami K, Uezono Y, Horishita T, Ogata J, Shiraishi M, Okamoto T, Terada T, Sata T. The effects of the tramadol metabolite o-desmethyl tramadol on muscarinic receptor-induced responses in xenopus oocytes expressing cloned m1 or m3 receptors. *Anesth Analg*. 2005; 101: 180–6.
52. Mostafa G, Mohamad MF, Bakry RM, Farrag WS. Effect of tramadol and ropivacaine infiltration on plasma catecholamine and postoperative pain. *J Am sci*. 2011; 7: 473–79.
53. Miyano K, Minami K, Yokoyama T, Ohbuchi K, Yamaguchi T, Murakami S, Shiraishi S, Yamamoto M, Matoba M, Uezono Y. Tramadol and its metabolite m1 selectively suppress transient receptor potential ankyrin 1 activity, but not transient receptor potential vanilloid 1 activity. *Anesth Analg*. 2015; 120: 790–8.
54. Isirdia-Espinoza MA, Pozos-Guillén A, Pérez-Urizar J, Chavarría-Bolaños D. Involvement of nitric oxide and atp-sensitive potassium channels in the peripheral antinoceptive action of a tramadol-dexketoprofen combination in the formalin test. *Drug dev res*. 2014; 75: 449–54.
55. Marincák R, Tóth BI, Czifra G, Szabó T, Kovács L, Bíró T. The analgesic drug, tramadol, acts as an agonist of the transient receptor potential vanilloid-1. *Anesth Analg*. 2008; 106: 1890–6.
56. Boderá P, Stankiewicz W, Zawada K, Antkowiak B, Paluch M, Kieliszek J, Kalicki B, Bartosiński A, Wawer I. Changes in antioxidant capacity of blood due to mutual action of electromagnetic field (1800 mhz) and opioid drug (tramadol) in animal model of persistent inflammatory state. *Pharmacol Rep*. 2013; 65: 421–8.
57. Bajic D, Berde CB, Commons KG. Periaqueductal gray neuroplasticity following chronic morphine varies with age: Role of oxidative stress. *Neuroscience*. 2012; 226: 165–77.
58. Sharifipour M, Izadpanah E, Nikkhoo B, Zare S, Abdolmaleki A, Hassanzadeh K, Moradi F, Hassanzadeh K. A new pharmacological role for donepezil: Attenuation of morphine-induced tolerance and apoptosis in rat central nervous system. *J Biomed Sci*. 2014; 21: 6.
59. Hassanzadeh K, Habibi-asl B, Farajnia S, Roshangar L. Minocycline prevents morphine indu-

ced apoptosis in rat cerebral cortex and lumbar spinal cord: A possible mechanism for attenuating morphine tolerance. *Neurotox Res.* 2011; 19: 649–59.

60. Popovic M, Janicijevic-Hudomal S, Kaurinovic B, Rasic J, Trivic S, Vojnovic M. Antioxidant effects of some drugs on immobilization stress combined with cold restraint stress. *Molecules.* 2009; 14: 4505–16.

61. (61) Zhang, Y Z, Qiu-Sheng P, Jing Z, Rong-Liang. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. *Basic clin pharmacol toxicol.* 2004; 95: 53–58

62. Ghoneim FM, Khalaf HA, Elsamanoudy AZ, Helaly AN. Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: Histological, immunohistochemical and biochemical study. *Int J Clin Exp Pathol.* 2014; 7: 7323.

63. Nna V, Akpan U, Osim E. Hyperprolactinemia contributes to reproductive deficit in male rats chronically administered pde5 inhibitors (sildenafil and tadalafil) and opioid (tramadol). *APJR.* 2016; 5: 381–6.

64. Lemarie A, Grimm S. Mutations in the heme b-binding residue of sdhc inhibit assembly of respiratory chain complex ii in mammalian cells. *Mitochondrion.* 2009; 9: 254–60.

65. Mohamed TM, Ghaffar HMA, El Husseiny RM. Effects of tramadol, clonazepam, and their combination on brain mitochondrial complexes. *Toxicol Ind Health.* 2015; 31: 1325–33.

66. Butterfield DA, Castegna A, Lauderback CM, Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in alzheimer's disease brain contribute to neuronal death. *Neurobiol Aging.* 2002; 23: 655–64.

67. Abdel-Zaher AO, Abdel-Rahman MS, ELwasei FM. Protective effect of nigella sativa oil against tramadol-induced tolerance and dependence in mice: Role of nitric oxide and oxidative stress. *Neurotoxicology.* 2011; 32: 725–33.

68. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid β -peptide-associated oxidative stress and brain protein modifications in the pathogenesis of alzheimer's disease and mild cognitive impairment. *Free Radic Biol Med.* 2007; 43: 658–77.

69. Kanter M. Nigella sativa and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. *Neurochem Res.* 2008; 33: 579.

70. Rabei HM. The immunological and histopathological changes of tramadol, tramadol/acetaminophen and acetaminophen in male albino rats: Comparative study. *The Egyptian Journal of Hospital Medicine.* 2011; 31: 1–56.

71. Alici HA, Ozmen I, Cesur M, Sahin F. Effect of the spinal drug tramadol on the fatty acid compositions of rabbit spinal cord and brain. *Biol Pharm Bull.* 2003; 26: 1403–6.

72. Ahmed MA, Kurkar A. Effects of opioid (tramadol) treatment on testicular functions in adult male rats: The role of nitric oxide and oxidative stress. *Clin Exp Pharmacol Physiol.* 2014; 41: 317–23.

73. Liu L-W, Lu J, Wang X-H, Fu S-K, Li Q, Lin F-Q. Neuronal apoptosis in morphine addiction and its molecular mechanism. *Int J Clin Exp Med.* 2013; 6: 540.

74. Atici S, Cinel L, Cinel I, Doruk N, Aktekin M, Akca A, Camdeviren H, Oral U. Opioid neurotoxicity: Comparison of morphine and tramadol in an experimental rat model. *Int J Neurosci.* 2004; 114: 1001–11.

75. Heidari Z, Mahmoudzadeh-Sagheb H, Kohan F. A quantitative and qualitative study of rat testis following administration of methadone and buprenorphine. *Int J High Risk Behav Addict.* 2012; 1: 12–5.

76. Azmy SM, El-Rahman SSA, Nada SA, Salam OMA, El-Yamany MF, Nassar NN. Does nicotine impact tramadol abuse? Insights from neurochemical and neurobehavioral changes in mice. *Neurotoxicology.* 2018.

77. Lees G. Inhibition of sodium-potassium-atpase: A potentially ubiquitous mechanism contributing to central nervous system neuropathology. *Brain Res Rev.* 1991; 16: 283–300.

78. Elwy AE-HM, Tabl G. Impact of tramadol and morphine abuse on the activities of acetylcholine esterase, Na⁺/K⁺-atpase and related parameters in cerebral cortices of male adult rats. *Electron physician.* 2017; 9: 4027.

79. de Souza Wyse AT, Streck EL, Worm P, Wajner A, Ritter F, Netto CA. Preconditioning prevents the inhibition of Na⁺, K⁺-atpase activity after brain ischemia. *Neurochem Res.* 2000; 25: 971–5.

80. Horvat A, Momic T, Petrovic S, Nikezic G, Demajo M. Selective inhibition of brain Na⁺, K⁺-atpase by drugs. *Physiol Res.* 2006; 55: 325.

81. Baghishani F, Mohammadipour A, Hosseinzadeh H, Hosseini M, Ebrahimzadeh-bideskan A. The effects of tramadol administration on hippocampal cell apoptosis, learning and

memory in adult rats and neuroprotective effects of crocin. *Metab Brain Dis.* 2018; 1–10.

82. Baghishani F, Mohammadipour A, Hosseinzadeh H, Hosseini M, Ebrahimzadeh-bideskan A. The effects of tramadol administration on hippocampal cell apoptosis, learning and memory in adult rats and neuroprotective effects of crocin. *Metab Brain Dis.* 2018; 33: 907–16.

83. Senay EC, Adams EH, Geller A, Inciardi JA, Munoz A, Schnoll SH, Woody GE, Cicero TJ. Physical dependence on ultram® (tramadol hydrochloride): Both opioid-like and atypical withdrawal symptoms occur. *Drug Alcohol Depend.* 2003; 69: 233–41.

84. Inass E, Hassan M, Fouad G, El-Komey F. Toxic effects of paroxetine on sexual and reproductive functions of rats. *Egyptian Journal of Hospital Medicine.* 2005; 21: 16–32.

85. Loughrey M, Loughrey C, Johnston S, O'Rourke D. Fatal hepatic failure following accidental tramadol overdose. *Forensic Sci Int.* 2003; 134: 232–3.

86. Elkhateeb A, El Khishin I, Megahed O, Mazen F. Effect of nigella sativa linn oil on tramadol-induced hepato-and nephrotoxicity in adult male albino rats. *Toxicol Rep.* 2015; 2: 512–9.

87. Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral U. Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model. *J Biosci.* 2005; 30: 245–52.

88. Awadalla EA, Salah-Eldin A-E. Histopathological and molecular studies on tramadol mediated hepato-renal toxicity in rats. *J Pharm Biol Sci.* 2015; 10: 90–102.

89. Boshra V. Evaluation of osteoporosis risk associated with chronic use of morphine, fentanyl and tramadol in adult female rats. *Curr drug saf.* 2011; 6: 159–63.

90. Garcia AN, Salloum IM. Polysomnographic sleep disturbances in nicotine, caffeine, alc-

ohol, cocaine, opioid, and cannabis use: A focused review. *Am j addict.* 2015; 24: 590–8.

91. Bohra MH, Kaushik C, Temple D, Chung SA, Shapiro CM. Weighing the balance: How analgesics used in chronic pain influence sleep? *Br j pain.* 2014; 8: 107–18.

92. Pattinson K. Opioids and the control of respiration. *Br J Anaesth.* 2008; 100: 747–58.

93. Panagiotou I, Mystakidou K. Non-analgesic effects of opioids: Opioids' effects on sleep (including sleep apnea). *Curr Pharm Des.* 2012; 18: 6025–33.

94. Walder B, Tramer M, Blois R. The effects of two single doses of tramadol on sleep: A randomized, cross-over trial in healthy volunteers. *Eur J Anaesthesiol.* 2001; 18: 36–42.

95. Hassanian-Moghaddam H, Kolahi A, editors. Tramadol intoxication/abuse: A new issue on high-access population. Sixth Annual Congress of Asia Pacific Association of Medical Toxicology; 2007.

96. Tjäderborn M, Jönsson AK, Hägg S, Ahlner J. Fatal unintentional intoxications with tramadol during 1995–2005. *Forensic Sci Int.* 2007; 173: 107–11.

97. Simonsen KW, Edvardsen HME, Thelander G, Ojanperä I, Thordardottir S, Andersen LV, Kriikku P, Vindenes V, Christoffersen D, Delaveris GJM. Fatal poisoning in drug addicts in the nordic countries in 2012. *Forensic Sci Int.* 2015; 248: 172–80.

98. Awadalla EA, Salah-Eldin A-E. Molecular and histological changes in cerebral cortex and lung tissues under the effect of tramadol treatment. *Biomed Pharmacother.* 2016; 82: 269–80.

99. Alizadeh-Ghamsari A, Dadpour B, Najari F. Pathological findings of tramadol on liver tissue in the cadaver referred to legal medicine organization of tehran 2008-2013. *I JMTFM.* 2016; 6: 59–64.