

Impact of tramadol and morphine abuse on the activities of acetylcholine esterase, Na⁺/K⁺-ATPase and related parameters in cerebral cortices of male adult ratsAbd El-Hamid Mohamed Elwy¹, Ghada Tabl²

¹ Ph.D., Assistant Professor, Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University, Tanta city-Gharbia Governorate- Egypt

² Ph.D., Zoology Department, Faculty of Science, Tanta University, Tanta city-Gharbia Governorate- Egypt

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Objective: To determine the effect of the most commonly abused drugs (tramadol and morphine), on acetylcholine esterase (AChE), Na⁺/K⁺-ATPase activities and related parameters, Na⁺ and K⁺ as biomarkers of neurotoxicity.

Methods: Tramadol - as a weak μ opioid receptor agonist- and morphine - as opiate analgesic drugs, were chosen for the present study. Four series of experimental animals were conducted for either tramadol or morphine: control series; repeated single equal doses (therapeutic dose) series; cumulative increasing doses series and delay (withdrawal) series (96 hours withdrawal period after last administration), at time period intervals 7, 14 and 21 days. Acetylcholine esterase (AChE), Na⁺/K⁺-ATPase activities and related parameters, Na⁺ and K⁺ were measured in cerebral cortices of experimental rats.

Results: Acetylcholine esterase (AChE) activity in the brain cerebral cortex increased after the administration of therapeutic repeated doses of either tramadol (20 mg/kg b.w.) or morphine (4 mg/kg b.w.) in different groups. The daily intraperitoneal injection of cumulative increasing dose levels of either tramadol 20, 40 and 80 mg/kg or morphine 4, 8 and 12 mg/kg revealed a significant increase in the mean of acetylcholine esterase activities. The withdrawal groups of either tramadol or morphine showed significant decreases in their levels. Na⁺/K⁺ ATPase activity in the brain cerebral cortex of either repeated therapeutic doses of tramadol (20 mg/kg) or morphine repeated therapeutic doses (4 mg/kg) for 21 consecutive days at different intervals 7, 14 and 21 days, induced a significant decrease in the levels of Na⁺/K⁺-ATPase in all groups. Withdrawal groups showed a significant decrease in Na⁺/K⁺-ATPase level. Furthermore, the daily intraperitoneal injection of cumulative increasing dose levels of either tramadol (20, 40 and 80 mg/kg b.w.) or morphine (4, 8 and 12 mg/kg b.w.) induced significant decreases in Na⁺/K⁺-ATPase levels in all studied groups. Regarding Na⁺ and K⁺, concentrations of either repeated therapeutic doses or cumulative increasing doses at different time intervals, showed different fluctuations in their levels.

Conclusion: The recorded data suggest that both drugs exert potent effects on AChE and Na⁺/K⁺-ATPase activities which could contribute to cerebral cortex malfunction including, memory deficits and the decline in cognitive function observed in chronic users.

Keywords: Morphine, Tramadol, Acetylcholine esterase (AChE), Na⁺/K⁺-ATPase

1. Introduction

Drug abuse is a worldwide problem. Drugs are very useful and inevitable if they are used properly but can be very harmful if they are abused. Tramadol and morphine are the most commonly used drugs of abuse. Tramadol (ultram) is a centrally acting analgesic drug with weak μ -opioid receptor agonist properties. Part of its analgesic effect is produced by inhibition of the reuptake of serotonin, and noradrenalin in the brain (1). Tramadol has a low affinity

Corresponding author:

Assistant Professor Dr. Abd El-Hamid Mohamed Elwy, Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University, Tanta city-Gharbia Governorate- Egypt.

Tel: 01223110312, 01013188644, And 01118607997, Email: elwyabdelhamid@gmail.com

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for opioid receptors and lacks selectivity towards the different receptor subtypes, and its analgesic potency is less than 10% that of morphine (2) and binds to m-opioid receptors with approximately 100 times less affinity than morphine (3). In several countries tramadol is becoming increasingly popular as a drug of abuse (4-6). Moreover, no official reports were made available in many Arab countries, since drug abuse is prohibited by legal and religious regulations. However, some adolescents are of the belief that, since alcohol is prohibited religiously, they can use other drugs with similar effect. Opioids such as morphine and heroin are well known to exert their effects by mimicking naturally occurring substances, termed endorphins (7). It is well known that morphine has primary activity at m-opioid receptors with some relative affinity for delta and kappa receptors (8). Exogenous opiates e.g. morphine, inhibit, by a feedback mechanism the firing of neurons that normally release the endogenous endorphins as neural transmitters. Thus, the endogenous endorphins accumulate and their concentrations increase at the nerve terminal, leading to an abnormal state in which there is only exogenous opiate present in the synapse to react with the target neuron. This corresponds to tolerance on abrupt cessation of exogenous opiate administration, or when naloxone is injected into the animal, the receptors are temporarily deprived of both endorphins and morphine. So, withdrawal symptoms result until endorphins of the neurons begin to be fired and are released at a normal rate. Morphine is available for oral use in standard and controlled release preparations. Due to first-pass metabolism, morphine is two to six folds less potent orally than parenterally. This is important to remember when converting a patient from parenteral to oral medication. There is wide variability in the first-pass metabolism, and the dose should be titrated to the patient's needs. Acetylcholine esterase (AChE) is an enzyme that catalyzes the breakdown of acetylcholine into choline and acetic acid, and it has function as neurotransmitters. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides. As AChE is the degradative enzyme of acetylcholine (ACh), it is responsible for the termination of cholinergic response in muscarinic and nicotinic brain ACh receptors (9). In the brain, normal activity of acetylcholine esterase is fundamental for the vital function of the brain, and changes in AChE activity are reported to be accompanied by apparent indication of neurobehavioral toxicity. Accordingly, this parameter can be used as a neurotoxicity marker in animals and humans (9). The well-known membrane-bound transport ATPases are Na^+ & K^+ -ATPase and Ca^{2+} & Mg^{2+} -ATPase. Na^+ & K^+ -ATPase transports Na^+ & K^+ and plays a central role in whole-body osmoregulation purposes. Interaction of environmental pollutants with ATPases however, evoked a good deal of benefit. The activities of Na^+/K^+ -ATPase in numerous tissues may be affected by different endogenous modulators. Moreover, this enzyme is probably under the effectiveness of various exogenous factors including some organic compounds of toxicological impact (10) and some drugs (11). The present study focuses on the neurotoxic effects accompanying therapeutic repeated equal single doses or cumulative increasing doses resulting from the abuse of either tramadol or morphine in the cerebral cortex of a mammalian experimental model. Tramadol, as weak μ opioid receptor agonist, and morphine, as opiate analgesic drugs were chosen for the present study.

2. Material and Methods

2.1. Animals

The experimental animals used in this study were male adult albino rats; they were obtained from the Breeding Unit of the Egyptian Organization for Vaccine and Biological Preparations, with initial body weight ranging from 130 - 140 g. All rats were kept under the same environmental conditions. The animals were fed ad Libitum with a standard diet and allowed free access of water. Animal experimentations were carried out in an ethical manner following guide lines for scientific research.

2.2. Drugs and chemicals

Tramadol (tramadol hydrochloride ampoules) was obtained from October Pharma Product, S.A.E. Morphine (sulphate ampoules) was obtained from Misr Co. Pharma. S.A.E. Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A).

2.3. Dose

In the present investigation, the dose used was based on the therapeutic levels for the two drugs, because the therapeutic dose enables to follow drugs at an average used dose. Subsequently, it is upgraded to other levels to follow up the dangerous effects of the two drugs. The adopted experimental dose level has been calculated as equivalent to the human therapeutic dose.

2.4. Experimental protocol

To evaluate the extent at which the tested drugs could affect some brain transmitters, four series of experiments were conducted: equal repeated therapeutic tramadol series (20 mg/kg); cumulative increasing doses of tramadol series

(20, 40, 80 mg/kg); morphine equal therapeutic repeated series (4 mg/kg) and cumulative increasing doses of morphine series (4, 8, 12 mg/kg). Each series was divided into five groups: control; 7th; 14th; 21st day groups and withdrawal group 96 hours from the last given dose according to Sepúlveda et al. (12). In all groups of either tramadol or morphine therapeutic equal repeated or cumulative increasing doses, eight rats were injected i.p. with tested daily dose for 21 consecutive days at time intervals on days 7, 14 and 21 of injection. Rats were euthanized at the end of each time interval on days 7, 14 and 21. At the end of each experiment, rats were euthanized quickly with the least disturbance by fast decapitation to avoid any substantial changes in brain transmitters. The brain was carefully removed and processed for the estimation of studied parameters under investigation.

2.5. Statistical analysis

Data were analyzed using F-test (ANOVA) by SPSS Version 9. Effects with a probability of $p < 0.05$ were considered to be significant.

3. Results

3.1. Acetylcholine esterase activities in different groups

Collective data of acetylcholine esterase activity in the cerebral cortex of experimental rats affected by continuous intraperitoneal injection of either tramadol or morphine, single equal repeated doses (therapeutic doses) and cumulative increasing doses at selected period intervals 7, 14 & 21 days and 96 hours' withdrawal period are given in Tables 1 & 2. Regarding the effect of repeated equal single doses of either tramadol (20 mg/kg) or morphine (4 mg/kg), data showed a significant increase of acetylcholine esterase activities in either groups treated with tramadol or morphine compared with control groups $p < 0.01$ and $p < 0.001$, respectively. Considering the withdrawal, the tramadol group showed insignificant changes compared to the control level ($p > 0.05$). In contrast, the withdrawal period of morphine showed a significant decrease ($p < 0.001$).

Table 1. Effect of tramadol consecutive repeated doses (therapeutic) 20 mg/kg on acetyl cholinesterase (AChE) activity nmol /min/g in cerebral cortices of male albino rats for 7, 14 and 21 days' time interval, as well as, the withdrawal effect.

Parameters	Control (mean±SE)	Duration (mean±SE)			Withdrawal (mean±SE)	F-value (ANOVA)
		7 days	14 Days	21 Days		
AChE (nmol /min/g)	14.888±0.136	15.438±0.125 ¹	15.900±0.113 ²	16.988±0.138 ²	14.713±0.16 ²	45.384 ²

1: $p < 0.01$; $p < 0.001$

Table 2. Effect of increasing tramadol doses, 20, 40 and 80 mg/kg on acetyl cholinesterase (AChE) activity nmol /min/g in cerebral cortex of male albino rat for 7, 14 and 21 days' time interval and withdrawal effect.

Parameters	Control (mean±SE)	Duration (mean±SE)			Withdrawal (mean±SE)	F-value (ANOVA)
		7 days	14 Days	21 Days		
AChE (nmol /min/g)	14.188±0.175	14.788±0.196 ¹	16.063±0.096 ²	16.813±0.181 ³	13.450±0.166 ²	67.821 ³

1: $p < 0.05$; 2: $p < 0.01$; 3: $p < 0.001$

3.2. Cumulative increasing doses of either tramadol (20, 40 & 80 mg/kg) or morphine (4, 8 & 12 mg/kg)

The present results demonstrated that the daily intraperitoneal injection at different dose levels of either tramadol (20, 40 & 80 mg/kg) or morphine (4, 8 & 12 mg/kg) produced a significant increase in the mean of acetylcholine esterase activities with a level of significance of $p < 0.05$ and $p < 0.001$, respectively. On the other hand, the withdrawal groups of either tramadol or morphine showed significant decrease with a level of significance of $p < 0.01$ and $p < 0.001$, respectively.

3.3. Na⁺ and K⁺-ATPase activity in different groups

Collective data of Na⁺ & K⁺-ATPase activity in the cerebral cortices of experimental rats affected by continuous intraperitoneal injection of either tramadol or morphine, single equal repeated doses and cumulative increasing doses at selected period intervals 7, 14, and 21 days and 96 hours' withdrawal period are given in Tables 3 & 4.

Table 3. Effect of morphine repeated doses (therapeutic), 4 mg/kg on acetyl cholinesterase (AChE) activity (nmol/min/g) in cerebral cortex of male albino rat for 7, 14 and 21 day intervals and withdrawal effect.

Parameters	Control (mean±SE)	Duration (mean±SE)			Withdrawal (mean±SE)	F-value (ANOVA)
		7 days	14 Days	21 Days		
AChE (nmol/min/g)	13.788±0.245	14.675±0.149 ¹	15.625±0.187 ²	16.638±0.138 ²	12.375±0.129 ²	88.737 ²

1: $p < 0.01$; 2: $p < 0.001$

Table 4. Effect of increasing morphine doses 4, 8 and 12mg/kg on acetyl cholinesterase (AChE) activity nmol/min/g in cerebral cortex of male albino rat for 7, 14 and 21 days and withdrawal effect.

Parameters	Control (mean±SE)	Duration (mean±SE)			Withdrawal (mean±SE)	F-value (ANOVA)
		7 days	14 Days	21 Days		
AChE (nmol/min/g)	14.088±0.160	14.613±0.099 ¹	16.313±0.130 ²	17.175±0.197 ¹	11.850±0.109 ²	208.658 ²

1: $p < 0.01$; 2: $p < 0.001$

3.4. Repeated equal single dose of either tramadol (20 mg/kg) or morphine (4 mg/kg)

The administration of either tramadol or morphine induced significant decrease in the level of Na⁺ and K⁺-ATPase in studied groups with a level of significance of $p < 0.01$ and $p < 0.001$, respectively. Considering day 7 of tramadol administration, there was insignificant change ($p > 0.05$). The withdrawal groups of both drugs showed significant decrease in Na⁺ & K⁺-ATPase ($p < 0.001$) compared to the control group.

3.5. Cumulative increasing doses of either tramadol (20, 40 & 80 mg/kg) or morphine (4, 8 & 12 mg/kg)

Data recorded for the administration of either tramadol or morphine at different dose levels (20, 40 & 80 mg/kg) and (4, 8 & 12 mg/kg) induced progressive significant decrease ($p < 0.001$) in Na⁺ & K⁺-ATPase levels in all studied groups except on the 7th day in the tramadol group. The withdrawal groups of both drugs showed significant decrease in Na⁺ & K⁺-ATPase ($p < 0.001$) as compared to the control group.

3.6. Na⁺ and K⁺ concentrations in different groups

Data of Na⁺ and K⁺ concentrations in the cerebral cortex were affected by continuous intraperitoneal injection of either tramadol or morphine. Single repeated doses and cumulative increasing doses at selected period intervals 7, 14 and 21 days and 96 hours' withdrawal period are given in Tables 5 & 6.

Table 5. Effect of tramadol repeated doses (therapeutic), 20 mg/kg on concentration of Na⁺/K⁺-ATPase μmole/Pi-min. fresh tissue in cerebral cortices of male albino rats for 7, 14 and 21 day intervals and withdrawal effect.

Parameters	Control (mean±SE)	Duration (mean±SE)			Withdrawal (mean±SE)	F-value (ANOVA)
		7 days	14 Days	21 Days		
Na ⁺ /K ⁺ -ATPase μmol/Pi-min	6.591±0.200	6.111±0.204	5.418±0.224 ¹	5.015±0.247 ¹	5.164±0.226 ¹	9.187 ¹

1: $p < 0.01$; 2: $p < 0.001$

Table 6. Effect of tramadol repeated doses (therapeutic), 20 mg/kg on concentration of Na⁺ mg/g and K⁺ mg/g in cerebral cortices of male albino rats for 7, 14 and 21 day intervals and withdrawal effect.

Parameters	Control (mean±SE)	Duration (mean±SE)			Withdrawal (mean±SE)	F-value (ANOVA)
		7 days	14 Days	21 Days		
Na ⁺ mg/g	3.738±0.178	3.900±0.230	4.150±0.247	4.450±0.254	4.263±0.146	1.744
K ⁺ mg/g	4.773±0.285	4.624±0.301	4.375±0.255	4.075±0.161	4.438±0.165	1.208

3.7. Repeated equal single dose of either tramadol (20 mg/kg) or morphine (4 mg/kg)

Tramadol administration produced insignificant change ($p > 0.05$) in Na⁺ and K⁺ concentrations in all studied groups. Morphine administration showed significant increase in Na⁺ concentration on days 14 and 21 ($p > 0.05$). In contrast morphine showed significant decrease in K⁺ levels on days 14 and 21 with significance level of $p < 0.05$ and $p < 0.001$, respectively. In addition, the withdrawal group showed insignificant changes in both Na⁺ and K⁺ levels after

tramadol administration ($p > 0.05$). On the other hand, morphine administration induced insignificant change in Na^+ level of the withdrawal group and significant decrease in K^+ level ($p < 0.05$). Within cumulative increasing doses of either tramadol (20, 40 & 80 mg/kg) or morphine (4, 8 & 12 mg/kg), the statistical evaluation of the repeated administration of tramadol at different dose levels, for 21 days at selected period intervals 7, 14 & 21 days on Na^+ concentration proved a significant increase on day 21 ($p < 0.05$) and there was significant decrease relation recorded when comparing the K^+ level on day 21 with the control group ($p < 0.01$). On the basis of increasing morphine doses (4, 8 & 12 mg/kg), Na^+ concentration showed significant increase on the 7th, 14th and 21st days ($p < 0.05$; $p < 0.01$; $p < 0.001$, respectively) but there was a significant decrease in K^+ level on the 14th and 21st days. Considering, the withdrawal group, after tramadol administration, the recorded data showed insignificant relation on Na^+ and K^+ levels when compared with control values ($p > 0.05$). But, the results recorded for the withdrawal morphine group showed a significant increase in Na^+ level and significant decrease on the K^+ level ($p < 0.01$). In conclusion tramadol or morphine administrations at different dose levels induced various fluctuations in Na^+ and K^+ concentrations of the cerebral cortex.

4. Discussion

Drug abuses that lead to dependence are major health problems worldwide. The major drugs that are abused such as stimulants, narcotics, sedatives, hypnotics and hallucinogens, may destroy tissues and cause much torture to individuals and societies as well as lead to death. Accordingly, drugs are very useful and essential if they are used properly but may be very harmful if they are abused. In the current study using adult male albino rats as an experimental model, tramadol as non-opioid analgesic and morphine as opiate analgesic drugs were chosen for the present study. The response of the brain tissues towards the two tested drugs under investigation was as follows; tramadol and morphine at tested dose levels and selected time periods on acetylcholine esterase (AChE); Na^+ & K^+ -ATPase and related parameters; sodium and potassium in brain tissues of albino rats was investigated. The present investigation focuses on the neurotoxic effects and biochemical changes accompanying the toxicity resulting from the misuse of these drugs in a mammalian experimental model. In addition, it throws some light on the influence of the withdrawal period of the drug toxicity. The selected doses were mainly dependent on the therapeutic values. The duration period was chosen according to periods previously adopted by several investigations that ranged from a single dose up to 21 days of the therapeutic dose (12, 13). With regard to the importance of these enzymes; acetylcholine esterase (AChE) and Na^+ & K^+ -ATPase for the proper functions of the brain tissues and nerve cells, the current study was undertaken in order to investigate the effects of tramadol and morphine at tested doses and selected time periods on both acetylcholine esterase (AChE) and Na^+ & K^+ -ATPase activities and the related parameters; sodium and potassium in rat brain tissues. Acetylcholine esterase (AChE) is the enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid. Acetylcholine esterase hydrolyzes ACh faster than the other choline esters. Moreover, in brain normal activity of acetylcholine esterase it is essential for the healthy function of the brain, and changes in AChE activity are reported to be accompanied by clear signs of neurobehavioral toxicity. Therefore, this parameter can be used as a neurotoxicity index in animals and humans (9). The central cholinergic neurotransmission in the brain is crucial for cognitive functions including memory and learning (14). Consequently, changes in central cholinergic activity will be of an important factor on cognitive functions. Moreover, inhibitors of brain acetylcholine esterase, such as donepezil and rivastigmine, which is used to treat the cognitive decline due to aging or Alzheimer's disease by increasing extracellular acetylcholine and the signaling neurotransmitter of the cholinergic system, would have an important impact (15, 16). This comes in agreement with (14) who reported that the central cholinergic activity possess an important role on cognitive functions. Therefore, the objective of the present study was to investigate the action of these two drugs under investigation; morphine and tramadol on the activities of brain acetylcholine esterase which is the key enzyme that terminates the action of acetylcholine at cholinergic synapses and is highly efficient in modulating the levels of extracellular acetylcholine and in regulating cholinergic neurotransmission (17). The present results demonstrated that the daily intraperitoneal administration of either tramadol or morphine over 7, 14 and 21 days at different dose levels (repeated therapeutic or incremental doses) produced a significant increase in the mean brain acetyl cholinesterase activity. The significant increase in brain acetyl cholinesterase (AChE) activity by these drugs would suggest a decrease in brain levels of ACh, which could explain in part, the cognitive and memory dysfunction in the users of tramadol or morphine. This comes in contact with Hosseini-Sharifabad et al. (18), who reported memory impairing action for tramadol. Consequently, it may lead to many problems such as bad social adaptation and decreasing productivity of work or may even lead to many deaths. In addition, the increased dose levels of both drugs did not induce more changes in most of the studied parameters. This can be attributed to the fact that, the long-term use of opioids can, however, result in tolerance and dependence. There are number of studies linking acute receptor desensitization to tolerance and dependence (19, 20). Furthermore, the results of the present investigation

come in accordance with Dang and Williams (21) who reported that tolerance and dependence result from long-term exposure to opioids, and there is growing evidence linking acute receptor desensitization to this more long-term process. Receptor desensitization encompasses a series of events leading to a loss of the receptors function. Balzan et al. (22) reported that the activities of Na^+ & K^+ -ATPase may be influenced by different endogenous modulators in numerous tissues. Moreover, Na^+ & K^+ -ATPase activity is decreased by toxic actions of normal neurotransmitters such as glutamate, which is the main cause of cell injury leading to death of neurons, and in various neurodegenerative disorders. Furthermore, Vasić et al. (10) reported that Na^+ & K^+ -ATPase enzyme may be under the influence of various exogenous factors including some organic compounds of toxicological effects, as well as some drugs. Consequently, its activities will decrease and the abnormal functioning of rat brain cerebral cortex may be the cause of many different types of neurological disorders. Furthermore, Lees (23) reported that this enzyme (Na^+ & K^+ -ATPase) supports the ionic homeostasis of the cells, maintenance of neuronal resting membrane potentials and propagation of neural impulses. Therefore, the abnormal function of this enzyme will lead to brain dysfunction due to cellular depolarization. In addition, along the same lines, the significant decreases in the activities of brain Na^+ & K^+ -ATPase recorded in the present study of either tramadol or morphine at different dose levels, may lead to cell injury and death of neurons, consequently, brain disorders. This is in line with earlier studies (22-25). Another explanation is that the drugs under investigation may exert their inhibiting effects by direct action on the brain enzyme protein itself. According to the present results, the significant change in Na^+ , K^+ -ATPase, which is well known as an integral membrane enzyme that activates the transport of Na^+ and K^+ ions against concentration gradients, may explain the fluctuations in Na^+ and K^+ concentrations in the present study. Since Na^+ and K^+ play a role in body functions including transmission of nerve signals, fluid balance and various chemical reactions. Abnormal functioning of brain Na^+ & K^+ -ATPase could be the cause of many different types of neurological disorders. This is in agreement with Horvat et al. (26) who suggested that depolarization of cell membrane causes abnormally excessive amounts of certain neurotransmitters to be released.

5. Conclusions

The findings of the present study suggest potent effects of the two tested drugs; tramadol and morphine on the activities of AChE and Na^+ & K^+ -ATPase. The actions of these drugs are contributed to the memory deficits and the decline in cognitive function in chronic users. As a consequence, the effects of both drugs could be a marker for drug-induced neurotoxicity. Establishment of psychological centers should be given particular attention to treat the victims newly fallen in the pitch of addiction (drugs of abuse) using the most up-to-date techniques and new treatment trends for treating our young victims from the dreadful use of these drugs. Additionally, help must be offered along with experience which will encourage victims to break from the shackles of addiction, and put an end to this fatal condition. Moreover, adequate guidance and advice on religious and ethical values should be delivered through visual and audible media. Furthermore, programs which are based on scientific evidence should be established. Fortunately, any devoted effort for the addict (victim) who searches for pleasure and relieving worry is worthwhile.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

Both authors contributed to this project and article equally. Both authors read and approved the final manuscript.

References:

- 1) Bloms-Funke P, Dremencov E, Cremers TI, Tzschentke TM. Tramadol increases extracellular levels of serotonin and noradrenaline as measured by in vivo microdialysis in the ventral hippocampus of freely-moving rats. *Neurosc Lett.* 2011; 490(3): 191-5. doi: 10.1016/j.neulet.2010.12.049. PMID: 21195741.
- 2) Grond S, Sablotzki A. Clinical pharmacology of tramadol. *Clin Pharmacokinet.* 2004; 43(13): 879-923. doi: 10.2165/00003088-200443130-00004. PMID: 15509185.

- 3) Minami K, Ogata J, Horishita T, Shiraishi M, Okamoto T, Sata T, et al. Intramuscular tramadol increases gastric pH during anesthesia. *Can J Anesth.* 2004; 51(6): 545-8. doi: 10.1007/BF03018395. PMID: 15197115.
- 4) Randall C, Crane J. Tramadol deaths in Northern Ireland: a review of cases from 1996 to 2012. *J Forensic Leg Med.* 2014; 23: 32-6. doi: 10.1016/j.jflm.2014.01.006. PMID: 24661703.
- 5) El-Hadidy MA, Helaly AM. Medical and Psychiatric Effects of Long-Term Dependence on High Dose of tramadol. *Subst Use Misuse.* 2015; 50(5): 582-9. doi: 10.3109/10826084.2014.991406. PMID: 25544109.
- 6) Tjäderborn M, Jönsson AK, Sandström TZ, Ahlner J, Hägg S. Non-prescribed use of psychoactive prescription drugs among drug-impaired drivers in Sweden. *Drug Alcohol Depend.* 2016; 161: 77-85. doi: 10.1016/j.drugaledep.2016.01.031. PMID: 26875672.
- 7) Howard B, Gutstein, Huda Akil. Opioid Analgesics. In: *Pharmacological Basis of Therapeutics*. 10th ed. Goodman & Gilman, Macmillan Comp. London, Canada, Toronto: 2001; 569-70.
- 8) Plumb DC. *Plumb's Veterinary Drug Handbook*. 7th ed. Stockholm, USA: Pharma Vet Inc; 2011.
- 9) Milatovic D, Gupta RC, Aschner M. Anticholinesterase toxicity and oxidative stress. *Scientific World Journal.* 2006; 6: 295-310. doi: 10.1100/tsw.2006.38. PMID: 16518518.
- 10) Vasić V, Jovanović D, Horvat A, Momić T, Nikežić G. Effect of Cd²⁺ and Hg²⁺ on the activity of Na⁺/K⁺-ATPase and Mg²⁺-ATPase adsorbed on polystyrene microtiter plates. *Anal Biochem.* 2002; 300(2): 113-20. doi: 10.1006/abio.2001.5405. PMID: 11779101.
- 11) Modi DN, Merchant MA. In vitro effects of aspirin and salicylate on erythrocytes: size and Na⁺/K⁺-ATPase activity. *Ind J Pharmacol.* 2003; 35: 27-31.
- 12) Sepúlveda J, Oliva P, Contreras E. Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine. *Eur J Pharmacol.* 2004; 483(2-3): 249-58. doi: 10.1016/j.ejphar.2003.10.037. PMID: 14729114.
- 13) Atici S, Cinel L, Cinel I, Doruk N, Aktekin M, Akca A, et al. Opioid neurotoxicity: comparison of morphine and tramadol in an experimental rat model. *Int J Neurosci.* 2004; 114(8): 1001-11. doi: 10.1080/00207450490461314.
- 14) Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. *Behav Brain Res.* 2011; 221(2): 555-63. doi: 10.1016/j.bbr.2010.11.058. PMID: 21145918.
- 15) Tan CC, Yu JT, Wang HF, Tan MS, Meng XF, Wang C, et al. Efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis.* 2014; 41(2): 615-31. doi: 10.3233/JAD-132690. PMID: 24662102.
- 16) Campos C, Rocha NB, Vieira RT, Rocha SA, Telles-Correia D, Paes F, et al. Treatment of cognitive deficits in Alzheimer's disease: a psychopharmacological review. *Psychiatr Danub.* 2016; 28(1): 2-12. PMID: 26938815.
- 17) Pohanka M. Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. *Int J Mol Sci.* 2014; 15(6): 9809-25. doi: 10.3390/ijms15069809. PMID: 24893223, PMCID: PMC4100123.
- 18) Hosseini-Sharifabad A, Rabbani M, Sharifzadeh M, Bagheri N. Acute and chronic tramadol administration impair spatial memory in rat. *Res Pharm Sci.* 2016; 11(1): 49-57. PMID: 27051432, PMCID: PMC4794937.
- 19) Ueda H, Inoue M, Matsumoto T. Protein kinase C-mediated inhibition of mu-opioid receptor internalization and its involvement in the development of acute tolerance to peripheral mu-agonist analgesia. *J Neurosci.* 2001; 21(9): 2967-73. PMID: 11312280.
- 20) Freye E, Latasch L. [Development of opioid tolerance -- molecular mechanisms and clinical consequences]. *Anesthesiol Intensivmed Notfallmed Schmerzther.* 2003; 38(1): 14-26. doi: 10.1055/s-2003-36558. PMID: 12522725.
- 21) Dang VC, Williams JT. Chronic morphine treatment reduces recovery from opioid desensitization. *J Neurosci.* 2004; 24(35): 7699-706. doi: 10.1523/JNEUROSCI.2499-04.2004. PMID: 15342737, PMCID: PMC3631536.
- 22) Balzan S, D'urso G, Ghione S, Martinelli A, Montali U. Selective inhibition of human erythrocyte Na⁺/K⁺-ATPase by cardiac glycoside and by mammalian digitalis like factor. *Life Sci.* 2000; 67(16): 1921-8. doi: 10.1016/S0024-3205(00)00779-7.
- 23) Lees GJ. Inhibition of sodium-potassium-ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. *Brain Res Brain Res Rev.* 1991; 16(3): 283-300. doi: 10.1016/0165-0173(91)90011-V. PMID: 1665097.

- 24) Brines ML, Dare AO, de Lanerolle NC. The cardiac glycoside ouabain potentiates excitotoxic injury of adult neurons in rat hippocampus. *Neurosci Lett.* 1995; 191(3): 145-8. doi: 10.1016/0304-3940(95)11577-J. PMID: 7644134.
- 25) 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(7): 971-5. doi: 10.1023/A:1007504525301. PMID: 10959493.
- 26) Horvat A, Momić T, Petrović S, Nikezić G, Demajo M. Selective inhibition of brain Na, K-ATPase by drugs. *Physiol Res.* 2006; 55(3): 325-38. PMID: 16083303.