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ROLE OF TNF-ALPHA, NF-KB AND SOME SELECTED INTERLEUKINS IN TRAMADOL RELATED MULTI-ORGANS DAMAGE IN RATS

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ABSTRACT

Introduction: Tramadol, an opioid medication used to treat moderate-moderately severe pain. The most common adverse effects of tramadol include nausea, dizziness, drowsiness and headache. The aim of study to investigate the effects of toxic doses of tramadol on liver, heart and brain of rats by utilizing diverse duration of exposure and to assess the concentration of tramadol in each of the above mentioned organs. **Method:** Thirty-five (35) adult Wistar Albino male rats; their age range (3-5) Months, weighing 200-250g were utilized in this study. They were obtained from the Animal House of the College of Pharmacy/Baghdad University. The animals were randomly allocated into five groups (7 animals each) as follows: (14 rats) and sub-divided into two sub-divisions: **A-** Rats IP injected with (0.5ml /250gm body weight) normal saline administered. **B-** Rats received twice-daily toxic dose (54mg/kg) tramadol for 8 days. **Results:** rats IP injected twice daily there were non-significant differences ($P>0.05$) in serum levels of -tumour necrosis factor-alpha, -nuclear factor kappa-light-chain-enhancer of activated B cells, -interleukin-10, -interleukin-1beta, -creatine kinase-MB, -aspartate aminotransferase, -alkaline phosphatase, and -aldehyde dehydrogenase, while, serum activity level of alanine aminotransferase enzyme activity was significantly decreased ($P<0.05$) in comparison with negative the corresponding serum activity level in negative control. significantly elevated ($P<0.05$) compared to its concentration in serum; furthermore, the concentration of the deliberated sub-acute toxic dose of tramadol was significantly elevated ($P<0.05$) in the heart compared to its concentration in either brain or liver tissue homogenate. **Conclusion:** the sub-acute toxic dose of tramadol (54mg/kg) had no effects on serum levels of various- cytokines and enzymes activity; this may point out that the concentration of the such toxic dose of tramadol in liver and heart tissues homogenate is not enough for causing damage to such tissues.

KEYWORDS: TNF-alpha, NF-κB, interleukins, Tramadol, Rats.

INTRODUCTION

The term toxicity indicates the degree to which a substance (toxin or poison) can harm humans or animals.The intensity of a toxic effect depends primarily on the concentration and persistence of the ultimate toxicant (active form) at its site of action which may react with the endogenous target molecule or critically alters the biological environment; this may initiate structural and/or functional alterations that result is toxicity.[1] Tramadol is a synthetic opioid of the benzenoid class. It can be used to treat moderate-moderately severe pain.[2] ***First***ly, it may work by binding to the μ-opioid receptor (as agonist), and ***secondly*** it may inhibit the reuptake of the neurotransmitters, serotonin and norepinephrine.[3, 4] Tramadol is used for moderate-severe pain. It can be given by mouth as plane tablet or as a modified-release preparation once or twice daily; also, such drug can be given IM, IV injection or infusion and rectally as a suppository.[5] Tramadol may produce fewer typical opioid adverse effects. While, at its overdosage, common symptoms may include lethargy, nausea, tachycardia, and agitation; in addition, seizures were also noted. Respiratory depression was seen in only small number of patients. The inhibitory effect of tramadol on monoamine reuptake, rather than its opioid effects, considered to result in much of its toxicity.[6] Tramadol should used with caution in patients with renal or hepatic impairment. Removal by hemodialysis reported to be minimal at 7%. Moreover, tramadol should used with care in patients

with a history of epilepsy or those susceptible to seizures.[7] It has reported that carbamazepine may have the ability to diminish the analgesic activity of tramadol by reducing its serum concentrations. Furthermore, the risk of seizures increased if tramadol used with drugs that have the potential to lower the seizure threshold.[8] Moreover, tramadol may inhibit the reuptake of noradrenaline and serotonin and may enhance serotonin release and thus, it may possibly interact with other drugs that enhance monoaminergic neurotransmission including lithium, tricyclic antidepressants, and SSRIs; furthermore, it should not be given to patients receiving MAOIs or within 14 days of their discontinuation.[9] Besides, as the metabolism of tramadol can be mediated by CYP2D6 isoenzyme, thus the use of specific inhibitors of this enzyme, such as quinidine, may increase concentrations of tramadol and lower concentrations of its active metabolite; although the clinical consequences of this effect are unclear.[10] The aim of study to investigate the effects of toxic doses of tramadol on liver, heart and brain of rats by utilizing diverse duration of exposure and to assess the concentration of tramadol in each of the above mentioned organs.

METHOD

Thirty-five (35) adult Wistar Albino male rats (the available sex); their age range (3-5) Months, weighing 200-250g were utilized in this study. They were obtained from the Animal House of the College of Pharmacy/Baghdad University. They were kept in a polypropylene-plastic cages (three animals/cage), under controlled conditions [temperature (25 ± 2^0 C), humidity and light/dark cycles]. The animals had free access to commercial pellet diet and water *ad libitum.* Rats were randomly allocated into five groups (7 animals each) as follows: **A- Controls:** Rats were I.P injected with (0.5ml /250gm body weight) NS twice daily for 8 days. Then the animals were euthanized by anesthetic ether on the day 9 after NS administration. This group served as negative control for Tramadol-treated animals. **B- Tramadol-treated animals**: Rats were IP injected with toxic dose of Tramadol (54 mg/kg/day) [11] twice daily for 8 days. Then the animals euthanized by anesthetic ether on the day 9 after drug administration. After 24 hrs of Ethanol administration, rats were euthanized by anesthetic diethyl ether and they underwent laparotomy then whole blood was collected by heart puncture and divided into two aliquots: Part 1: Whole blood put into polyethylene gel tube and left to clot then centrifuged at 3500 rpm for 15 minute to obtain serum, which was separated and stored at -40 Cº until the time of analysis. Serum utilized for the estimation of TNF-α, NF-κB, IL-10, IL-1β, CK-MB, ALT, AST, and ALDH. Part 2: whole blood put into anticoagulant tubes and was used for the determination of Ethanol concentration. Besides, after euthanization of rats by anesthetic diethyl ether, the Tramadol- treated animals underwent laparotomy then blood was collected by heart puncture and put into polyethylene gel tube. Blood samples were left to clot then centrifuged at 3500 rpm for 15 minute to obtain serum, which was separated and stored at -40 Cº until the time of analysis. Serum was utilized for the estimation of TNF-α, NF-κB, IL-10, IL-1β, CK-MB, ALT, AST, and ALDH; and for the determination of Tramadol concentrations. Five hundred (500) milligrams of liver, heart, and brain tissue were obtained from each male rat and then each was homogenized as well as deproteinized with 400µl of acetonitrile and 500µl of methanol. The resulted homogenate was vortexed for 1 min, the suspension of each tissue was centrifuged, filtered and evaporated to dryness at 45°C using vacuum oven.The resulted dry material was reconstituted with 100µl of the mobile phase, and centrifuged again. Then 30 µl of the obtained supernatant was directly injected into the HPLC. The samples were not diluted before HPLC measurement. Unknown contents of Nefopam and Tramadol were interpolated from the respective standard curves prepared previously.[12] Statistical Package for the Social Sciences (SPSS) 20.0.0, Minitab 17.1.0, and Graph Pad Prism 7.0 software package used to make the statistical analysis. Data were expressed as the mean values, mean± standard deviation (SD) of samples. The significance of differences between the mean values between drug-treated animals of same group with controls was calculated using unpaired Student's T-test. Furthermore, the statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA). Differences were considered statistically significant for *P*-value less than 0.05.

RESULTS

Table (1) showed that the TNF-α level in serum of rats IP injected with toxic twice daily dose (54mg/kg) of Tramadol for 8 days was non-significantly different compared to control rats ($P>0.05$).

Table 1: Effects of toxic dose of Tramadol (54mg/kg) twice daily for 8 days (sub-acute toxicity) on serum TNF-α level in rats.

Groups	Mean ± standard deviation (SD) of serum TNF- α levels
Control (n=7)	15.205 ± 1.275
Tramadol-treated (54mg/kg) (n=7)	18.227 ± 6.192

n= Number of animals.

Table (2) showed that in group of rats IP injected with toxic dose of Tramadol (54 mg/kg/day) twice daily for 8 days, serum NF-κB levels were reduced; however its level was non-significantly different (*P*>0.05) compared to the corresponding serum levels in control group of animals. The NF-κB levels being respectively, 418.951 ± 59.732 and 479.940 ± 82.026.

Table (2P): Effects of IP dose of Tramadol (54mg/kg) twice daily injected for 8 days (sub-acute toxicity) on serum NF-κB levels in rats.

Groups	Mean ± standard deviation (SD) of serum NF-κB levels
Control (n=7)	479.940 ± 82.026
Tramadol-treated (54mg/kg) (n=7)	418.951 ± 59.732

- n= Number of animals.

Intraperitoneal injection with toxic dose of Tramadol (54 mg/kg/day) twice daily for 8 days to rats produced a non-significant difference in the serum level of IL-10 compared to control animals (*P*>0.05). The serum levels of IL-10 being respectively, 19.783 ± 8.702 and 28.194 ± 14.557. Table 3.

Table (3): Effects of IP dose of Tramadol (54mg/kg) injected twice daily for 8 days (sub-acute toxicity) on serum IL-10 levels in rats.

Groups	Mean ± standard deviation (SD) of serum IL-10 levels
Control (n=7)	28.194 ± 14.557
Tramadol-treated (54mg/kg) (n=7)	19.783 ± 8.702

- N= Number of animals.

Table 4 showed in rats IP injected with toxic dose of Tramadol (54 mg/kg/day) twice daily for 8 days, the serum IL-1β level was reduced however, the intended serum level was a non-significantly different compared to the corresponding level in serum of control group (*P*>0.05); the levels being respectively, 20.623 ± 39.049 and 5.184 ± 4.625.

Table 4: Effects of IP dose of Tramadol (54mg/kg) injected twice daily for 8 days (sub-acute toxicity) on serum IL-1β levels in rats.

Groups	Mean ± standard deviation (SD) of serum IL-1β levels
Control (n=7)	5.184 ± 4.625
Tramadol-treated (54mg/kg) (n=7)	2.540 ± 0.348

- n= Number of animals.

Table 5 showed that the serum isoenzyme CK-MB activity level in rats IP injected with toxic dose of Tramadol (54 mg/kg/day) twice daily for 8 days was non-significant different (*P*>0.05) compared to the corresponding serum levels of the deliberated marker in control rats; the activities were respectively, 1.141 ± 0.757 and 0.916 ± 0.550.

Table 5: Effects of IP dose of Tramadol (54mg/kg) twice daily injected for 8 days (sub-acute toxicity) on serum CK-MB levels in rats.

Groups	creatine kinase myocardial band (CK-MB) pg/ml
Control N=7	0.916 ± 0.550
Tramadol-treated (54mg/kg) N=7	1.141 ± 0.757

- Each value represents Mean ± SD.
- N= Number of animals.

Serum ALT activity levels in rats IP injected with the toxic dose (54 mg/kg/day) of Tramadol twice daily for 8 days was significantly reduced (*P*<0.05) compared to the corresponding serum levels in control rats; the levels being respectively, 2.599 ± 1.727 and 5.900 ± 1.804. Table 6.

Table 6: Effects of IP dose of Tramadol (54mg/kg) twice daily injected for 8 days (sub-acute toxicity) on serum ALT levels in rats.

Groups	Alanine aminotransferase (ALT) pg/ml
Control N=7	5.900 ± 1.804
Tramadol-treated 54mg/kg N=7	2.599 ± 1.727 *

- Each value represents Mean ± SD.
- *: Significant difference (P<0.05) compared to control group.
- N= Number of animals.

Table 7 showed that serum AST activity in rats IP injected with toxic dose (54 mg/kg/day) of Tramadol twice daily for 8 days was non-significantly different (*P*>0.05) compared to the corresponding serum activity in control rats; serum enzyme activity levels being respectively, 2.300 ± 0.592 and 2.201 ± 0.739.

Table 7: Effects of IP dose of Tramadol (54mg/kg) twice daily injected for 8 days (sub-acute toxicity) on serum ALT levels in rats.

Groups	Aspartate aminotransferase (AST) Pg/ml
Control N=7	2.201 ± 0.739
Tramadol-treated 54mg/kg N=7	2.300 ± 0.592

- Each value represents Mean ± SD.
- N= Number of animals.

Serum ALP activity level in rats IP injected with a toxic twice daily dose of Tramadol (54 mg/kg/day) for 8 days was non-significantly different (*P*>0.05) compared to the corresponding serum enzyme activity level in control rats; the levels being respectively, 28.603 ± 3.278 and 27.921 ± 5.493. Table 8.

Table 8: Effects of IP dose of Tramadol (54mg/kg) twice daily injected for 8 days (sub-acute toxicity) on serum ALP levels in rats.

Groups	Alkaline phosphatase (ALP) pg/ml
Control N=7	27.921 ± 5.493
Tramadol-treated (54mg/kg) N=7	28.603 ± 3.278

- Each value represents Mean ± SD.
- N= Number of animals.

Table 9 showed that serum ALDH in rats IP injected with toxic twice daily dose of Tramadol (54 mg/kg/day) for 8 days was non-significantly different compared to control animals (*P*>0.05); the levels being respectively, 2.738 ± 1.355 and 3.638 ± 1.600.

Table 9: Effects of IP toxic dose of Tramadol (54mg/kg) twice daily injected for 8 days (sub-acute toxicity) on serum ALDH levels in rats.

Groups	Aldehyde dehydrogenase (ALDH) pg/ml
Control N=7	0.916 ± 0.550
Tramadol-treated (54mg/kg) N=7	1.141 ± 0.757

- Each value represents Mean ± SD.
- N= Number of animals.

Table 10 showed that after 8 days of IP injection of twice daily toxic dose (54 mg/kg/day) of Tramadol to rats, the intended drug's concentration in serum was 39.431 ± 0.923 µg/500 ml; while, in brain was 71.782 ± 6.768 µg/500 mg; and in liver was 87.483 ± 18.457 µg/500 mg; and in heart was 104.531 ± 8.258 µg/500 mg. Moreover, there the concentration of toxic dose (54mg/kg) of tramadol twice daily injected for 8 days (sub-acute toxicity) in serum was significantly reduced compared to the corresponding concentration of the intended drugs in brain, liver, and heart (*P*<0.05). Besides, by comparing the concentration of IP dose (54mg/kg) of Tramadol twice daily injected for 8 days in brain, table 3-21 and figure 3-10 showed that the concentration of Tramadol

in brain was non-significantly differ to those concentration in liver (P>0.05) ; while it was significantly reduced with respect to heart (P<0.05). Additionally, the concentration of toxic dose (54mg/kg) of Tramadol twice daily injected for 8 days (sub-acute toxicity) in liver was significantly reduced compared to the corresponding concentration in heart (P<0.05).

Table 10: Concentrations of IP toxic dose (54mg/kg) of Tramadol twice daily injected for 8 days (sub-acute toxicity) in serum, brain, liver and heart of rats.

	Concentrations of toxic dose of Tramadol twice daily injected for 8 days (sub-acute toxicity)			
	Serum µg/ml	**Brain µg/mg**	**Liver µg/mg**	**Heart µg/mg**
Tramadol-treated rats (54mg/kg)	39.431 ± 0.923	71.782 ± 6.768 * a	87.483 ± 18.457 * a	104.531 ± 8.258 * b
Number of animals	7	7	7	7

- *: Significantly different (P<0.05) compared to Mean±SD Tramadol concentration in serum.
- Values with non-identical small letter (a, and b) are significantly different (P<0.05).

DISCUSSION

In the current study, levels of TNF-α was not statistically significant (P>0.05) in serum of rats IP injected with sub-acute toxic dose of Tramadol (54mg/kg) compared to the corresponding serum levels of controls; the finding of this study was in agreement with previous study performed by others.[13] The results obtained from the current study showed that, the serum level of NF-κB was lower in group of rats IP injected with 54mg/kg Tramadol twice daily for 8 days (sub-acute toxic dose) compared to control animals, however this reduction was not statistically significant (P>0.05). The finding of the current study was in tune with previous results obtained by others.[14] In sub-acute model of toxicity which was performed in the current study by utilizing toxic dose of Tramadol (54 mg/kg) IP injected to rats twice daily for 8 days, the serum level of IL-10 was lower in Tramadol-treated rats compared to the corresponding serum level in controls, however this reduction was not statistically significant (P>0.05); such finding was in agreement with the previous study of others.[15] In the current study, serum level of IL-1beta was lower in group of rats IP injected with 54mg/kg Tramadol twice daily (sub-acute toxicity module) compared to control, however this reduction was not statistically significant (P>0.05). Such finding was similar to the result obtained by others; where, they found that Tramadol depressed the expression of mRNA levels of IL-1beta.[14] The results obtained from this study showed that the serum isoenzyme CK-MB activity level in rats IP injected with toxic dose of Tramadol (54 mg/kg/day) twice daily for 8 days was non-significant different (P>0.05) compared to the corresponding serum CK-MB level in control rats. The results of this study are inconsistent with those performed by others[16]; where, authors reported that Tramadol has the potential to cause deleterious effects to major organs including the liver. In the current study, the serum ALT activity levels in rats IP injected with the toxic dose (54 mg/kg/day) of Tramadol twice daily for 8 days was significantly reduced (P<0.05) compared to the corresponding serum levels in control rats. Moreover, the serum AST activity in rats IP injected with toxic dose (54 mg/kg/day) of Tramadol twice daily for 8 days was non-significantly different (P>0.05) compared to the corresponding serum activity in control rats. Furthermore, serum ALP activity level in rats IP injected with a toxic twice daily dose of Tramadol (54 mg/kg/day) for 8 days was non-significantly different (P>0.05) compared to the corresponding serum enzyme activity level in control rats. The results of this study are inconsistent with the corresponding results of others.[16]

The present study showed that serum ALDH in rats IP injected with toxic twice daily dose of Tramadol (54 mg/kg/day) for 8 days was non-significantly different compared to control animals (P>0.05). There was no previous study concerning the effect of sub-acute toxic dose of Tramadol on serum ALDH activity; thus, we could not have a chance for comparing the results of this study with others. In the current study, Tramadol concentration after IP injected twice daily for 8 days (sub-acute toxic model) was significantly reduced (P<0.05) in serum compared to its concentration in brain, liver, and heart. Moreover, higher concentration of sub-acute toxic dose of Tramadol (54mg/kg), was observed at day 9 of Tramadol treatment in the heart, then liver, and then brain tissues homogenate; and the concentration of Tramadol in the heart tissue homogenate was significantly higher (P<0.05) compared to the rest of tissue homogenates. Furthermore, the concentration of the intended sub-acute toxic dose of Tramadol in the heart tissue homogenate was 2.7 times-, in liver was 2.2 times- and it was 1.8 times- higher in brain tissue when each tissue homogenate was compared to the concentration of Tramadol in serum.[16]

CONCLUSION

The sub-acute toxic dose of tramadol (54mg/kg) had no effects on serum levels of various- cytokines and enzymes activity; this may point out that the concentration of the such toxic dose of tramadol in liver and heart tissues homogenate is not enough for causing damage to such tissues.

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